CHAPTER 4

CHANGES OF PIGMENTS AND COLOR IN SARDINE (*SARDINELLA GIBBOSA*) AND MACKEREL (*RASTRELLIGER KANAGURTA*) MUSCLE DURING ICED STORAGE

4.1 Abstract

Changes in pigments and color of sardine and mackerel muscles during iced storage were investigated. When the storage time increased, a gradual increase in pH was observed. The total extractable pigment and heme iron content decreased ($P<0.05$), while the non-heme iron content tended to increase throughout storage. The soret band of myoglobin decreased with the concomitant decrease in redness index (a^*/b^* ratio) when the storage time increased, suggesting the destruction of the heme protein. A blue shift of myoglobin observed in both species, coincided with a slight increase in metmyoglobin content and was associated with the darkening of meats caused by the oxidation of myoglobin. Myoglobin extractability of sardine and mackerel muscle with NaCl solution and distilled water during iced storage was carried out. Myoglobin was removed to a greater extent with increasing in washing cycle and the higher amount of myoglobin removed was found in sample washed with NaCl solution, compared with that washed with distilled water. Higher myoglobin removal resulted in a lower redness index of washed mince. Therefore, myoglobin extracting efficiency depended on fish species, muscle types, storage time and washing process.

4.2 Introduction

Myoglobin is a globular heme protein localized in red muscle fibers. Myoglobin concentration generally depends on species, breed, sex and age of animal, training and nature of

nutrition, muscular activity, oxygen availability, blood circulation and muscle type as well as the way the meat is treated (Postnikova *et al*., 1999; Livingston and Brown, 1981; Giddings, 1974). Myoglobin has been known to be a major contributor to the color of muscle, depending upon its derivatives and concentration (Postnikova *et al*., 1999; Faustman *et al*., 1992). The stability of myoglobin also affects the color of meat (Chen, 2003; Chanthai *et al*., 1998; Tajima and Shikama, 1987; Suzuki and Kisamori, 1984). Hemoglobin is lost rather easily during handling and storage, while myoglobin is retained by the muscle intracellular structure (Livingston and Brown, 1981). Therefore, color changes in meat are mainly due to the reaction of myoglobin with other muscle components, especially myofibrillar proteins (Hanan and Shaklai, 1995).

During the handling and storage of fish, a number of biochemical, chemical and microbiological changes occur, leading to the discoloration (Pacheco-Aguilar *et al*., 2000; O'Grady *et al*., 2001; Faustman *et al*., 1992). Discoloration of tuna during frozen storage is caused by the formation of metmyoglobin (Haard, 1992). This phenomenon can be influenced by many factors such as pH, temperature, ionic strength and oxygen consumption reaction (Renerre and Labas, 1987). Metmyoglobin formation is positively correlated with lipid oxidation (Lee *et al*., 2003a; Chan *et al*., 1997). Myoglobin and other heme compounds at high concentration in red meats functioned as prooxidants in muscle tissue (Love, 1983). Furthermore, metmyoglobin formed the cross-linking with myosin in the presence of hydrogen peroxide (Hanan and Shaklai, 1995).

Generally, both heme proteins in fresh fish can be removed during the washing process, leading to increased whiteness of the flesh. However, heme proteins become less soluble as the fish undergo deterioration. Chen (2003) reported that ice or frozen storage decreased the myoglobin extracting efficiency in washed milkfish due to the insolubility of myoglobin by the oxidation of myoglobin to form metmyoglobin. As a consequence, the surimi produced from the unfresh fish is more likely to be discolored, especially dark-fleshed fish species. Due to the shortage of lean fish, which are commonly used for surimi production, more attention has been given to dark-fleshed fish, such as sardine and mackerel as the raw material for surimi. After capture, the fish are normally kept in ice prior to unloading and during this stage discoloration of muscle can occur and binding of pigments to muscle can also take place. However, no

information regarding the changes in the pigments and in the color of the muscle of sardine and mackerel caught in Thailand has been reported.

Thus, this study aimed to investigate the changes in the pigments and in the color of sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) meat during the extended iced storage.

4.3 Materials and Methods

Chemicals

Bathophenanthroline disulfonic acid was purchased from Sigma (St. Louis. MO, USA). Sodium chloride, trichloroacetic acid and iron standard solution were obtained from Merck (Damstadt, Germany). Sodium dithionite was purchased from Riedel (Seeize, Germany). Disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Fluka (Buchs, Switzerland). All chemicals were of analytical grade.

Fish samples

Sardine (*Sardinella gibbosa*) with an average weight of 55-60 g and mackerel (*Rastrelliger kanagurta*) with an average weight of 85-90 g were caught from Songkhla-Pattani Coast along the Gulf of Thailand during March and April, 2004. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 2 h. Whole fish were immediately washed and kept in ice with a fish/ice ratio of 1:2 (w/w). The fish were placed and distributed uniformly between the layers of ice. The box containing fish and ice was kept at 4°C for 15 days. To maintain the ice content, melted ice was removed and replaced with an equal amount of ice every 2 days. During storage, 10 fish were randomly taken as the composite sample at 0, 3, 6, 9, 12 and 15 days for analyzes. The fish samples were filleted and manually excised into ordinary and dark muscles. The muscles were kept on ice during preparation and analysis.

pH determination

The pH of fish muscle was measured as described by Benjakul *et al*., (1997). Fish muscle was homogenized using an IKA Labortechnik homogenizer (Selangor, Malaysia) with 10 volumes of deionized water (w/v), and the pH was measured using a pH meter (Cyberscan 500, Singapore).

Color measurement

Color of dark and ordinary muscle from both species was determined by measuring the *L*, a** and *b** value using a colorimeter (Juki Corp, Tokyo, Japan). Redness index (*a*/b*)* of meat was determined as described by Chen *et al*. (1997).

Myoglobin analysis

The myoglobin content was determined by direct spectrophotometric measurement as described by Benjakul and Bauer (2001). A chopped sample of flesh (2 g) was weighed into a 50 ml polypropylene centrifuge tube and 20 ml of cold 40 mM phosphate buffer, pH 6.8, were added. The mixture was homogenized at 13,500 rpm for 10 sec, followed by centrifuging at 3,000 $\times g$ for 30 min at 4 $^{\circ}$C using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). The supernatant was filtered with Whatman No. 1 filter paper. The supernatant (2.5 ml) was added with 0.2 ml of 1% (w/v) sodium dithionite to reduce the myoglobin. The absorbance was read at 555 nm using a UV-1601 spectrophotometer (Shimadzu, Japan). Myoglobin content was calculated from the millimolar extinction coefficient of 7.6 and a molecular weight of 16,110 (Gomez-Basauri and Regenstein, 1992). The myoglobin content was expressed as mg/g sample.

The absorption spectra scanning of extracted myoglobin (without sodium dithionite) was performed by a V-530 UV/VIS double beam spectrophotometer (Jasco, Tokyo, Japan). The spectra were recorded from 250 to 750 nm at the scanning rate of 1,000 nm/min using 40 mM phosphate buffer, pH 6.8 as a blank.

Total pigment analysis

The total pigment content was determined according to the method of Lee *et al*., (1999). Flesh (2 g) was mixed with 9 ml of acid acetone (90% acetone, 8% deionized water and 2% HCl). The mixture was macerated with a glass rod and allowed to stand for 1 h at room temperature. The extract was filtered with a Whatman No. 42 filter paper, and the absorbance was read at 640 nm against an acid acetone blank. The total pigments were calculated as hematin using the following formula:

$$\text{Total pigment (ppm)} = A_{640} \times 680.$$

The total pigment content was expressed as mg/100 g sample.

Determination of heme iron content

Heme iron content was determined as described by Benjakul and Bauer (2001). The sample solution was prepared by the method previously mentioned for myoglobin content determination. The total heme pigment content was determined by direct spectrophotometric measurement at 525 nm. Heme iron was calculated based on myoglobin, which contains 0.35% iron (Gomez-Basauri and Regenstein, 1992). The heme iron content was expressed as mg/100 g sample.

Determination of non-heme iron content

The non-heme iron content was determined according to the method of Schricker *et al*. (1982). The chopped sample (1.0 g) was weighed into a screw cap test tube and 50 ml of 0.39% (w/v) sodium nitrite were added. A mixture (4 ml) of 40% trichloroacetic acid and 6 M HCl (ratio of 1: 1 (v/v), prepared freshly) was then added. The tightly capped tubes were placed in an incubator shaker at 65°C for 22 h and then cooled at room temperature for 2 h. The

supernatant (400 μl) was mixed with 2 ml of the non-heme iron color reagent, a mixture of bathophenanthroline disulfonic acid, double-deionized water and saturated sodium acetate solution at a ratio of 1:20:20 (w/v/v) (prepared freshly). After vortexing and standing for 10 min, the absorbance was measured at 540 nm. The non-heme iron content was calculated from iron standard curve. The iron standard solution (400 μl), with the concentration ranging from 0 to 2 ppm, was mixed with 2 ml of the non-heme iron color reagent. The concentration of non-heme iron was expressed as mg/100 g sample.

Metmyoglobin content

The analysis of metmyoglobin content was performed as described by Lee *et al*. (1999). The sample solution was prepared in the same manner with that for heme iron determination. The supernatant was subjected to absorbance measurement at 700, 572, and 525 nm. The percentage of metmyoglobin was calculated using the following equation (Krzywicki, 1982):

$$\%\text{Metmyoglobin} = \left\{ 1.395 - \left[(A_{572} - A_{700}) / (A_{525} - A_{700}) \right] \right\} \times 100.$$

Myoglobin extractability and color of washed mince

To prepare fish mince, fish fillets were minced to uniformity using a mincer (a diameter of 4 mm). The mince was subjected to washing with 1 or 2 cycles using distilled water or NaCl solution (0.2% NaCl (w/v) for sardine and 0.5% NaCl (w/v) for mackerel minces, respectively) (Chaijan *et al*., 2004). The mixtures of mince and washing media at a ratio of 1:3 (w/v) were stirred gently for 5 min at 4°C and centrifuged at 1,000×*g* for 5 min using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). Wash water was collected and extractable myoglobin content was determined. Redness index (*a**/*b**) of washed mince was determined according to the method of Chen *et al*. (1997).

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test (Steel and Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 8.0 for windows, SPSS Inc., Chicago, IL).

4.4 Results and Discussion

Changes in pH

A gradual increase in pH was obtained in mackerel muscle during 15 days of iced storage (Figure 11). For sardine, the pH remained constant in the first 12 days and increased sharply in pH on day 15. The differences in pH changes between the two species might be due to the differences in buffering capacity of muscle, which was presumably greater in sardine. The increase in pH was postulated to be due to an increase in volatile bases produced by either endogenous or microbial enzymes. Benjakul *et al*. (2002) reported that the decomposition of nitrogenous compounds caused an increase in pH in fish flesh. However, Ababouch *et al*. (1996) found that muscle pH of sardine (*Sardina pilchardus*) increased from 6.24 to 6.55 during iced storage up to 11 days. Increase in the pH of the muscle of two species of sardine, *S. pilchardus* and *Sardinops sagax caerulea*, during storage in ice were reported (Pacheco-Aguilar *et al*., 2000; Nunes *et al*., 1992; El Marrakchi *et al*., 1990;). Bennour *et al*. (1991) reported that the pH value of mackerel (*Scomber scombus*) varied from 5.95 to 6.24 during iced storage for 12 days.

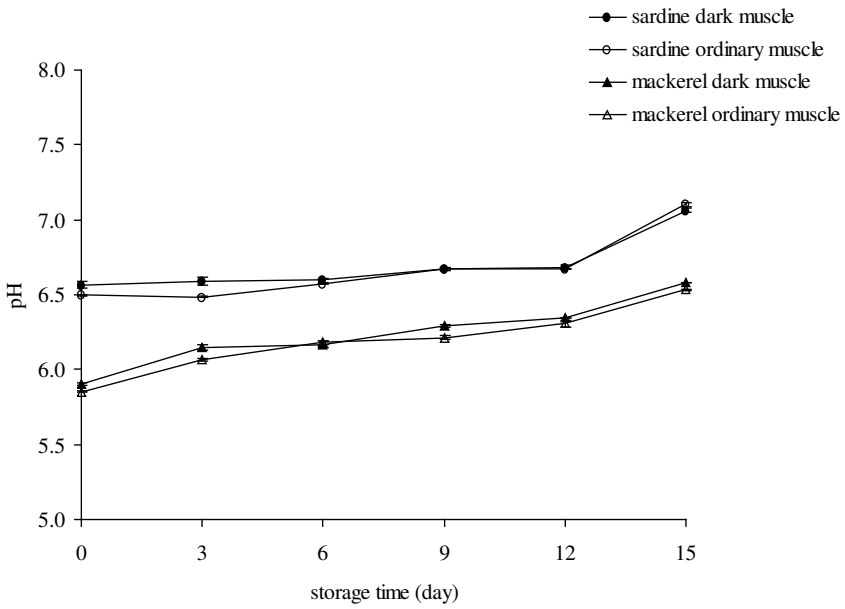


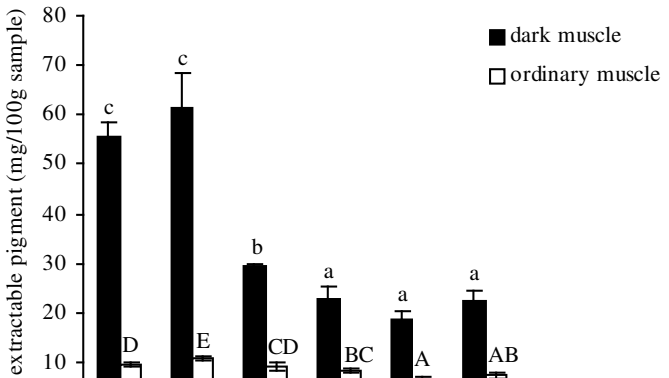
Figure 11. Changes in pH of sardine and mackerel muscles during iced storage. Bars indicate standard deviations from triplicate determinations.

Therefore, the changes in pH of sardine and mackerel muscle in this study, which might be different from other studies reported, depended on a variety of factors such as species, fishing ground, feeding of the fish, storage temperature and buffering capacity of meat (Pacheco-Aguilar *et al*., 2000). Sikorski *et al*. (1990) reported that the change in pH depended also on the liberation of inorganic phosphate and ammonia due to the enzymatic degradation of ATP. From the result, the initial pH between two species was different. Sardine muscle had higher pH than mackerel muscle. The activity of enzymes converting glycogen into lactic acid might be different between two species. Lactic acid, generated in anoxic conditions from glycogen, is the principal factor in lowering the *post-mortem* pH in the fish muscles (Sikorski *et al*., 1990). During the first 3 days, dark muscle of both species had slightly higher pH than ordinary muscle. Lawrie (1992) reported that red muscles were relatively deficient in the enzymes which convert glycogen into lactic acid. These features caused their *post-mortem* pH to be higher than that of white muscles. However, no marked differences in pH were found between ordinary and dark muscles of the two species after 6 days of storage.

Total extractable pigment

Dark muscle from sardine and mackerel contained much greater pigment content than ordinary muscle (P<0.05) (Figure 12A, B). The total pigment contents in dark and ordinary muscle of sardine were 55.67 and 9.60 mg pigment/100 g sample, respectively. For mackerel, values of 38.74 and 9.40 mg/100 g were found for dark and ordinary muscle, respectively. Kisia (1996) reported that sardine and mackerel contained more dark muscle fibers, and more mitochondria, myoglobin, fats, glycogen and cytochromes, than did white fleshed-fish species. The values obtained in sardine and mackerel were much lower than those reported for the yellowfin tuna (*Neothunnus macropterus*) light and dark muscle, which were 92 and 1,360 mg/100 g, respectively (Brown, 1961). Total extractable pigment content in all samples gradually decreased as the storage time increased (P<0.05). At day 15, the total pigment content of sardine dark and ordinary muscles decreased by 60% and 24%, respectively, compared with those obtained in fresh muscle. For mackerel muscle, the total pigment content of dark and ordinary muscles decreased by 60% and 18%, respectively, compared with those obtained at day 0 of storage. The result indicated that pigment possibly underwent oxidation or denaturation during storage, leading to the higher pigment content remaining or bound in the muscle. The result is in accordance with Chen (2003) who reported that myoglobin extracting efficiency in milkfish decreased with increasing iced storage time. Insolubility and binding of oxidized myoglobin to the muscle resulted in less removal of myoglobin during washing. From the result, it was noted that total pigments in dark muscle became less extractable, compared with ordinary muscle, with increasing storage time. This was possibly associated with the higher lipid oxidation in dark muscle, which had a high fat content. Hultin and Kelleher (2000a) reported that dark muscle, which contained a much greater lipid content and prooxidants, was more susceptible to oxidation than ordinary muscle.

(A)



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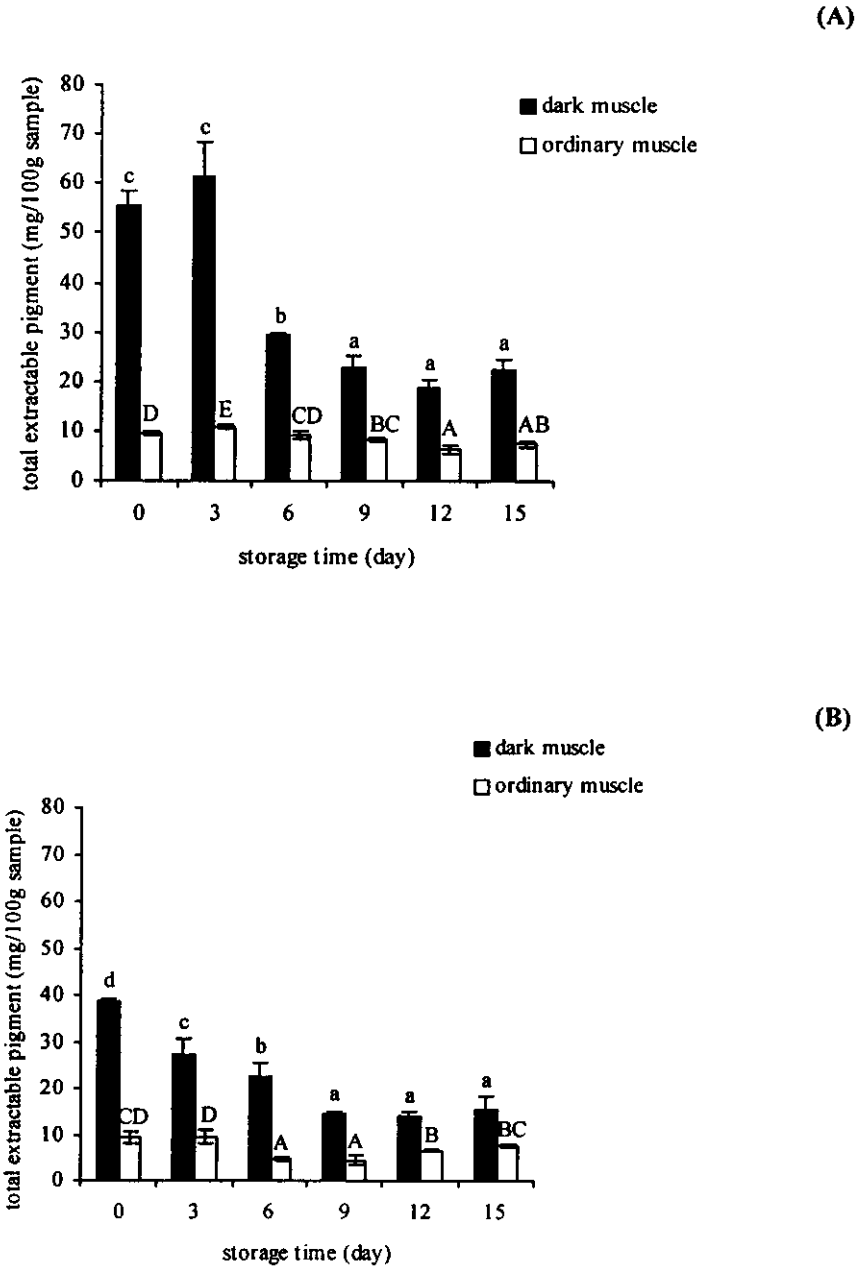

Figure 12. Changes in total extractable pigment of sardine (A) and mackerel (B) muscles during iced storage. Bars indicate standard deviations from triplicate determinations. Different letters under the same species and muscle types indicate significant differences ($P<0.05$).

Changes in heme iron content

Dark muscle comprised higher heme iron content than ordinary muscle in both species (Figure 13A). Sardine dark muscle had the greater heme iron content (9.16 mg/100 g) than that of mackerel (5.62 mg/100 g). Conversely, lower heme iron content was observed in sardine ordinary muscle (3.36 mg/100 g), compared with that of mackerel (4.68 mg/100 g). Presence of larger amounts of iron in the dark muscle reflected both higher content of hemoglobin and myoglobin as well as mitochondrial iron containing enzymes (Dulavik *et al.*, 1998). In general, the heme iron constituted 25-44% of the total iron in the fish (Fisher and Deng, 1977). Lue *et al.* (1981) reported that mackerel contained 1.2 mg iron/100 g edible portion, whereas Gomez-Basauri and Regenstein (1992) found that mackerel fillets had a total iron content of 0.7-0.9 mg/100 g and 46-65% of the iron in fillets was heme iron. Short-bodied mackerel (*Restrelliger brachysoma*) contained 0.8 mg heme iron/100 g edible portion (Kongkachuichai *et al.*, 2002). From the result, heme iron content most likely correlated with total pigment content (Figure 12). Thus, heme pigments were the major source of iron in both sardine and mackerel muscle. During the first 3 days of storage, the heme iron content increased markedly. Thereafter, heme iron content decreased ($P<0.05$) as the storage time increased (Figure 13). This might be due to the higher soluble heme pigment in fresh meat caused by autolysis. This might contribute to the greater extractability of heme pigments. The decreased heme iron content observed with extended storage time was presumed to be due to the release of free iron from heme. As a result, lower heme iron content was retained. Additionally, the lowered heme pigment extractability with increasing storage time also resulted in the lower iron content in heme extracted. Benjakul and Bauer (2001); Gomez-Basauri and Regenstein (1992) reported that the decrease in heme iron content in the muscle was inversely related to non-heme iron content.

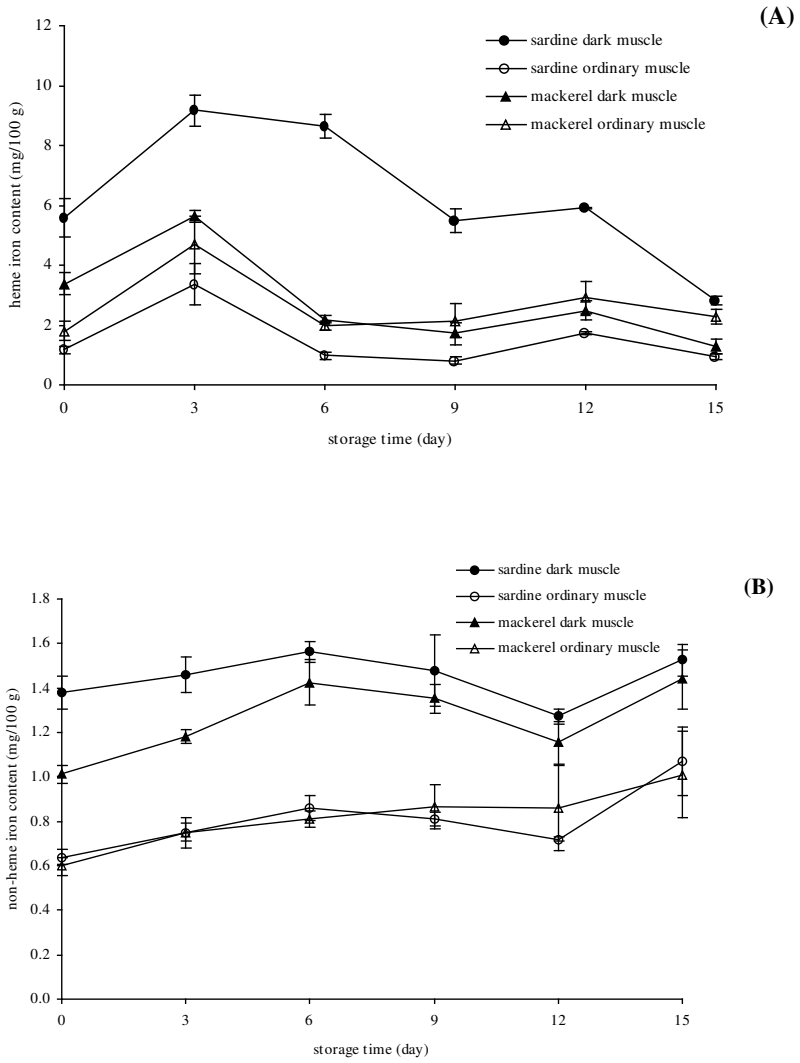

Figure 13. Changes in heme iron content (A) and non-heme iron content (B) of sardine and mackerel muscle during iced storage. Bars indicate standard deviations from triplicate determinations.

Changes in non-heme iron content

The changes in non-heme iron content in both sardine and mackerel muscles are shown in Figure 13B. At day 0, non-heme iron found in fresh samples were 1.01-1.38 and 0.60-0.64 mg/100g for dark muscle and ordinary muscle, respectively. Generally, dark muscle contained higher amount of non-heme iron than ordinary muscle. Hazell (1982) reported that iron

was distributed between five main components, including insoluble fraction, ferritin, hemoglobin, myoglobin and a low-molecular-weight fraction. Schricker *et al*. (1982) reported that total iron, heme iron and non-heme iron concentrations were significantly different between species and muscle types. The differences may relate to inherent differences in residual blood between white and red muscles in normal postmortem muscle (Schricker *et al*., 1982). During iced storage, non-heme iron content tended to increase with the increasing time up to 6 days. At day 12, the decrease in non-heme iron content in all samples was observed, which was coincidental with the slight increase in heme iron content. The released iron might bind tightly with the muscle components, which were denatured and easily interacted with those free irons. However, the non-heme iron content increased to a high extent at day 15. This might be due to the much more release of free iron from the muscle which was excessively degraded. Muscle proteins underwent degradation with increasing storage times in ice. Benjakul *et al*. (2003) reported the marked increase in protein hydrolysis, especially myosin heavy chain in lizardfish during extended iced storage. The results suggested that the heme pigment or other iron containing proteins were possibly denatured with increasing storage time, resulting in the release of iron. The denaturation of those components possibly contributed to the increase in non-heme iron content (Decker and Hultin, 1990a; 1990b). Decker and Hultin (1990a; 1990b) reported that the deterioration of subcellular organelles, e.g. the mitochondria, and the release of cytochrome *c*, could be responsible for the increase in soluble hemin.

Formation of metmyoglobin

The formation of metmyoglobin in dark and ordinary muscles of sardine and mackerel is shown in Table 6. No differences in metmyoglobin content were found in sardine dark muscle during 12 days of storage ($P>0.05$). For mackerel dark muscle, the marked increase in metmyoglobin was found at day 9. In fresh meat, reducing substances like NAD^+ or FAD^+ are endogenously produced, and they are responsible for the constant reduction of the brown-gray metmyoglobin or the purple myoglobin (Eder, 1996). Metmyoglobin reductase remained in the muscle might reduce metmyoglobin to other forms. The sharp increase in metmyoglobin formation with extended storage time suggested that myoglobin underwent more oxidation

(Benjakul and Bauer, 2001). The inactivation of enzymes which maintain the reduced state of hemoproteins was also presumed to increase the formation of metmyoglobin (Benjakul and Bauer, 2001).

Table 6. Metmyoglobin formation (%) in sardine and mackerel muscles during iced storage

Storage time	Sardine		Mackerel	
(days)	dark	ordinary	dark	ordinary
0	71.82±0.12a*	70.93±1.43b	70.70±3.51ab	69.32±0.94a
3	69.80±0.20a	68.14±0.32a	69.31±0.28a	75.17±0.54b
6	70.40±0.63a	75.99±1.22c	72.82±0.32bc	73.81±0.98b
9	70.70±1.25a	76.34±0.34c	74.25±0.08c	74.89±0.58b
12	69.83±2.12a	76.05±0.59c	80.06±0.26d	70.78±1.49a
15	76.61±0.61b	75.66±0.11c	78.90±0.60d	70.86±0.26a

Values are given as mean ± SD from triplicate determinations.

*Different letters in the same column indicate significant differences (P<0.05).

From the result, the metmyoglobin formation in ordinary muscle of both species tended to increase more rapidly than dark muscle. This might be due to the lower metmyoglobin reductase in ordinary muscle. It has been known that metmyoglobin reductase is a component of red blood cells and can be found in fish muscle (Al-Shaibani *et al*., 1977). Since some bloods were retained in the muscle, especially dark muscle, residual activity of this enzyme could be present and resulted in the retardation of color deterioration. Distribution and localization of myoglobin in both muscles might also be different, leading to the different susceptibility of myoglobin to oxidation. Many factors have been known to increase myoglobin oxidation, including pH, salt concentration and species (Trout, 1990). Surprisingly, the metmyoglobin formation in mackerel ordinary muscle was lowered at the end of storage time (day 12 and 15). This might be due to the rapid deterioration and more degradation of subcellular organelles including the mitochondria from this muscle, resulting in the release of pyridine nucleotides such as NAD(P)H that might involve in metmyoglobin reduction. The metmyoglobin reducing

capacity in post rigor muscle depended upon the availability of substrate, cofactors (e.g. pyridine nucleotide) and associated enzymic activities (e.g. dehydrogenase) (Giddings, 1974). Giddings (1974) reported that myoglobin was a diffusible cytosolute in the muscle cells and may be localized in close proximity to mitochondria. The generation of cytosolic NADH was essential for metmyoglobin reduction (Watts *et al*., 1966). Those free reducing substances reacted with metmyoglobin, leading to the reduction of metmyoglobin. Due to the extended storage, microorganisms especially psychrophilic bacteria might grow on the samples and possibly reduced metmyoglobin to some extent. Faustman *et al*. (1990) reported that in the presence of high levels of bacterial contamination, meat may display red color, which appeared to coincide with an increase in pH. Faustman *et al*. (1990) also found that inoculation of fluorescent pseudomonads at high levels in ground beef homogenates stored at 4°C converted metmyoglobin to oxymyoglobin or its derivatives.

Changes in absorption spectra and redness index

The strong absorption of myoglobin from both species was located in the blue region (350-450 nm) or soret band (data not shown). Swatland (1989) reported that the soret bands in meat for deoxymyoglobin, oxymyoglobin and metmyoglobin were 434, 416 and 410 nm, respectively. Higher peaks were observed in dark muscles, compared with those from ordinary muscles. With the same muscle, sardine showed the higher peak than mackerel. From the result, the soret peak in all samples decreased when the storage time increased. This disappearance of the soret absorption band indicated the destruction of the heme protein. Baron *et al*. (2002) reported that heme protein degradation was monitored by changes in the soret absorption band, known to be very sensitive to detachment of the porphyrin moiety from the globin.

No changes in absorption maxima in the soret region of myoglobin extracted from sardine and mackerel muscles were observed during the first 3 days of iced storage. Thereafter, a slight blue shift was observed in both dark and ordinary muscle of both species with increasing storage time (Table 7). A blue shift coincided with the slight increase in metmyoglobin in samples with increasing storage time. Antonini and Brunori (1971) reported that a blue shift from 418 to 409 nm was observed when sperm whale oxymyoglobin was changed to

metmyoglobin. From these results, it was presumed that during iced storage, the degradation and oxidation of heme proteins in both sardine and mackerel muscle occurred with varying degrees.

Table 7. Changes in absorption maxima (nm) in the soret region of myoglobin extracted from sardine and mackerel muscles during iced storage

Storage time	Sardine		Mackerel	
(days)	dark	ordinary	dark	ordinary
0	407	406	408	408
3	407	406	408	408
6	405	405	407	405
9	405	405	406	405
12	405	404	406	405
15	405	404	406	405

The redness index (*a*/b** ratio) of sardine and mackerel muscles decreased when the storage time increased (Figure 14), suggesting the darkening of meat most likely caused by the changes in pigments, mainly myoglobin. This ratio was used as an index of apparent change in redness (Chen *et al*., 1997) and used to evaluate the discoloration in tuna meat during storage (Lee *et al*., 2003b). At day 0, redness index of dark muscle was higher than that of ordinary muscle. Boulianne and King (1998) showed a strong positive correlation between total pigment concentration and a* value. The decrease in redness index was associated with the darkening of meats, resulting from the formation of metmyoglobin (Table 6) and was also coincidental with the disappearance of the soret absorption band as well as the shift of soret peak (Table 7). Faustman *et al*. (1992) reported that the saturation of red color in meat is directly related to myoglobin concentration. Fleming *et al*. (1991) reported that dark coloration in meat was also associated with the total pigment concentration. Thus, the changes in redness index can be used as the index of pigment changes of sardine and mackerel muscles.

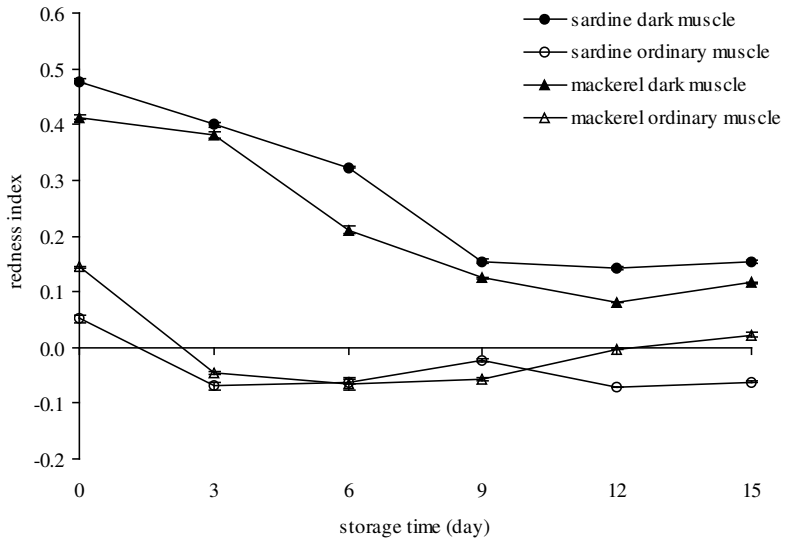

Figure 14. Changes in redness index of sardine and mackerel muscle during iced storage. Bars indicate standard deviation from triplicate determinations.

Effect of iced storage on myoglobin extractability and color of washed mince

Figure 15 shows the myoglobin extractability of sardine and mackerel muscle with NaCl solution and distilled water during iced storage. Myoglobin was removed to a great extent with increasing washing cycle for both dark and ordinary muscle from both species. Greater amount of myoglobin extracted was found in sample washed with NaCl solution, compared with that washed with distilled water. NaCl could weaken the interaction or bonding between myoglobin and muscle, leading to the release of myoglobin from the muscle. As Na^{+} and Cl^{-} are bound to acidic and basic amino acid residues, intermolecular ionic bonds among protein molecules are broken (Lee, 1992).

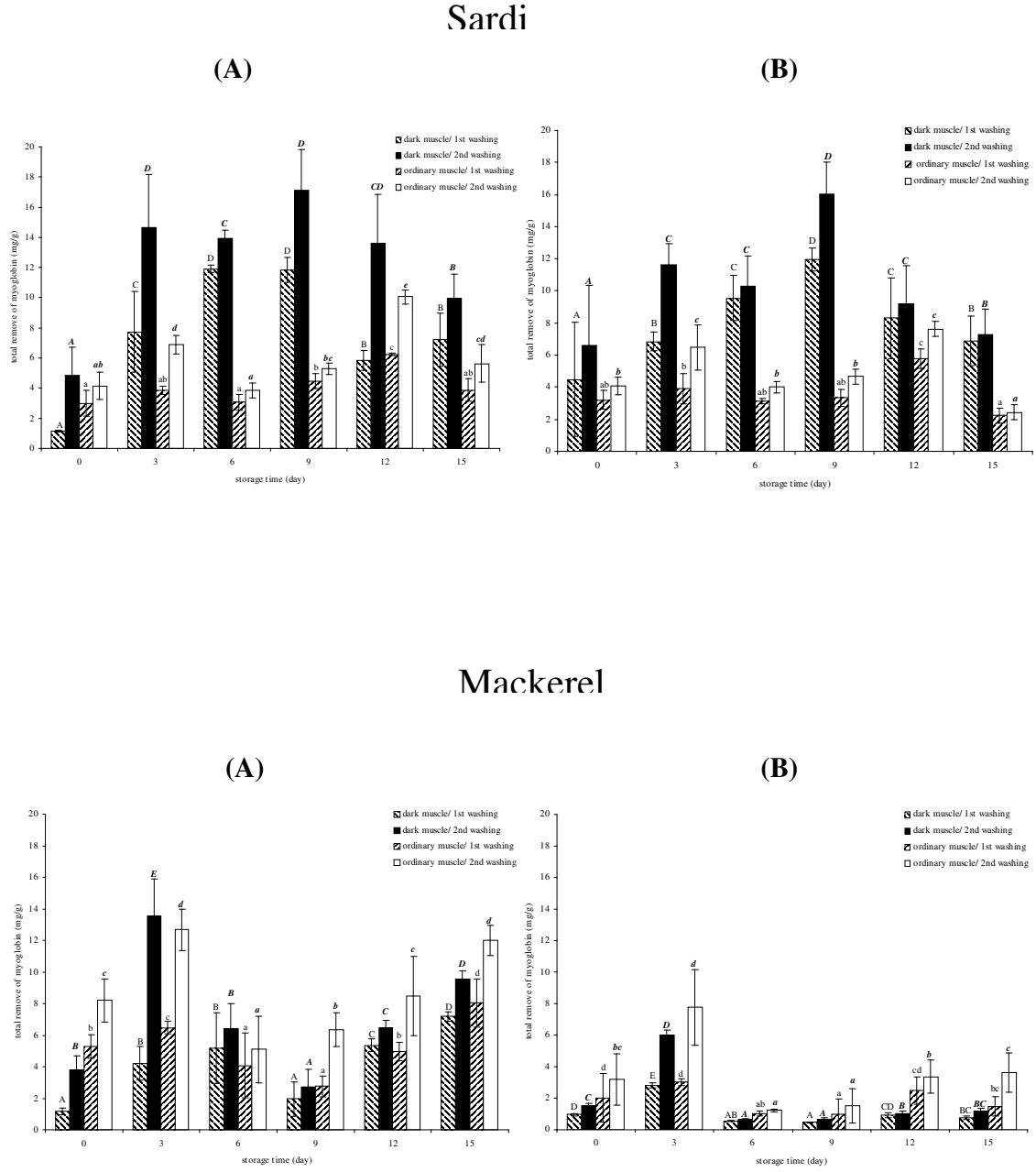

Figure 15. Changes in myoglobin extractability of sardine and mackerel mince during iced storage. The samples were extracted with NaCl solution (A) and distilled water (B). Bars indicate standard deviation from triplicate determinations. Different letters under the same muscle types and washing cycles indicate significant differences (P <0.05).

For sardine dark muscle washed with NaCl solution and distilled water, the extractable myoglobin increased with increasing storage time up to 9 days ($P<0.05$). Thereafter, the decrease in myoglobin extractability was observed. For ordinary muscle, the removal of myoglobin increased gradually up to 12 days and the marked decrease was found at day 15. The increase in myoglobin removal was possibly due to the increased degradation of muscle proteins, leading to the enhanced efficiency in myoglobin removal from the disintegrated muscle. The decrease in myoglobin removal at the last day (day 15) was mostly due to the loss in myoglobin extractability caused by its denaturation. The presence of lipid oxidation products may alter myoglobin through covalent modification (Faustman *et al*., 1999) and could alter myoglobin redox stability (Lynch and Faustman, 2000).

The extractable myoglobin of dark and ordinary muscles of mackerel washed with NaCl solution and distilled water increased after the first 3 days. The decrease was observed during 6-9 days of storage. Subsequently, the marked increase in myoglobin removal up to 15 days. After 3 days of storage, partial degradation of muscle might facilitate the extraction process of reduced myoglobin with the low content of oxidized form. Myoglobin became less soluble as the storage time increased, possibly due to the tight association of myoglobin with the muscle protein induced by oxidation process. Thereafter, the increase in myoglobin extractable was observed at day 12 and 15. This might be due to the degradation of muscle protein, resulting in the weakened muscle structure, in which more myoglobin could be extracted from the muscle. Chen (2003) reported that ice storage decreased the myoglobin extracting efficiency in washed milkfish due to the insolubility of myoglobin. At the same time of storage, NaCl showed the greater efficiency in myoglobin removal, compared with distilled water. Two washing cycles also resulted in the higher myoglobin removal than one cycle of washing. The result indicated that the myoglobin extractability depended on myoglobin characteristics, species and muscle types, washing media and washing cycle.

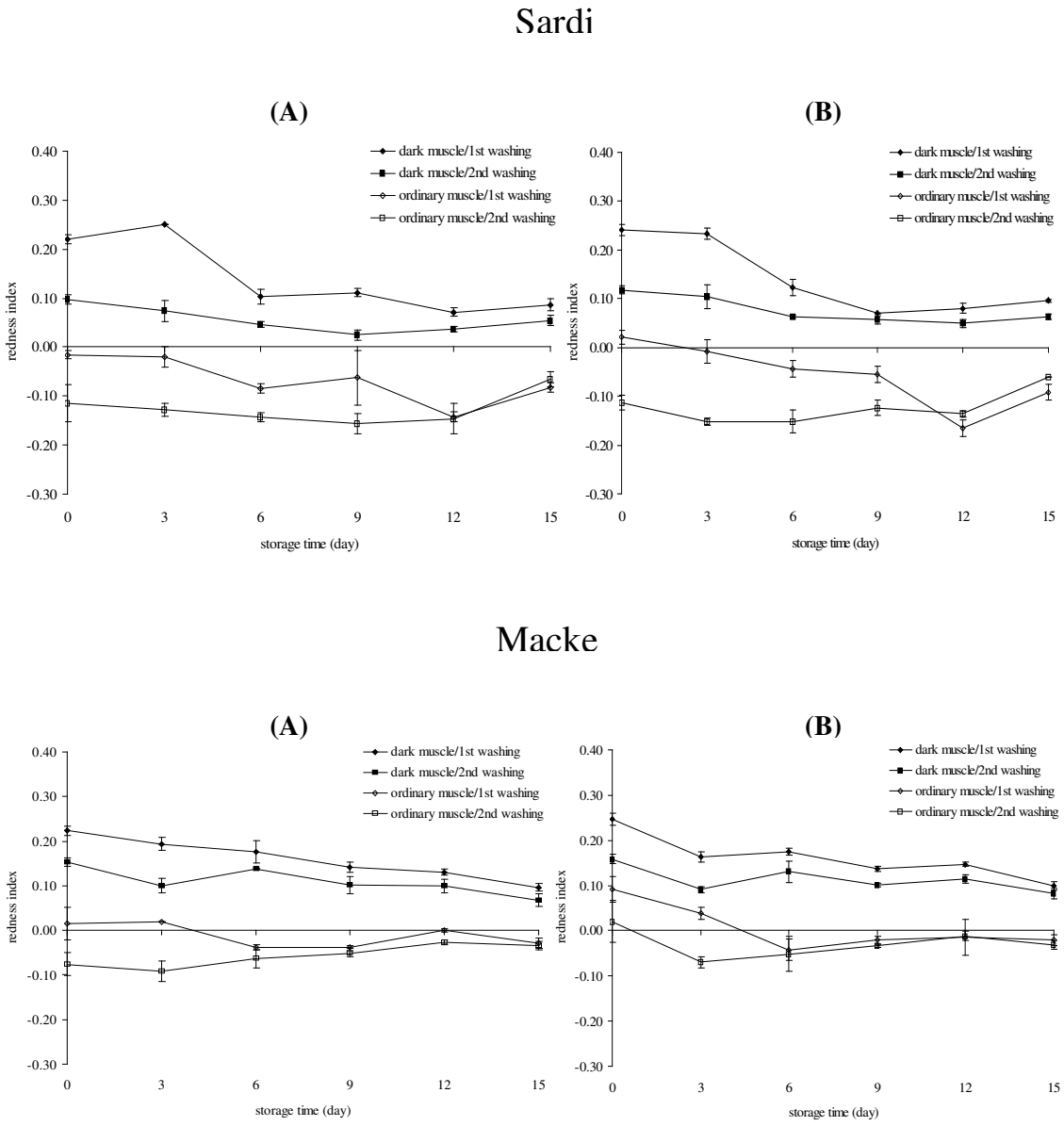

Figure 16. Changes in redness index of sardine mince and mackerel mince washed with NaCl solution (A) and distilled water (B) during iced storage. Bars indicate standard deviation from triplicate determinations.

Redness index of washed mince was lower than that of mince (Figure 14). The result suggested that some pigments, especially myoglobin and hemoglobin were removed, leading to the lowered redness. The redness index of washed mince either with NaCl solution or distilled water from both species decreased during the first 6 days of iced storage (P<0.05)

(Figure 16). This indicated that the discoloration of muscles was mainly caused by oxidation of myoglobin and the less extractability of pigments. Generally, the redness index of mince washed with 2 washing cycles was lower than those from 1 washing cycle, which was in accordance with myoglobin content removed (Figure 15).

Due to the superior gel forming ability of sardine to mackerel, the former species was used for further study.

4.5 Conclusion

Extended storage of sardine and mackerel in ice caused the oxidation and denaturation of pigment, mainly myoglobin, leading to the discoloration and less extractability of myoglobin. During storage, non-heme iron was released, which might accelerate the oxidation process in the muscle. Those changes showed the detrimental effect on color and pigment removal from the muscle of both species.