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Development of Yellow Discoloration in Sawai (*Pangasianodon hypophthalmus*) Muscle due to Lipid Oxidation

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ABSTRACT: In this study, we investigated the impact of lipid oxidation on the discoloration of Sawai (*Pangasianodon hypophthalmus*) lipids and proteins. Sawai microsomes, liposomes, and salt-soluble myofibrillar proteins were prepared and subjected to lipid oxidation process. The results revealed that the levels of thiobarbituric acid-reactive substances, yellowness (as indicated by b^* values), and pyrrole compounds increased when Sawai liposomes and microsomes were oxidized using iron and ascorbate. Meanwhile, the levels of free amines decreased, particularly as the iron content (25～100 μM) and incubation time (0～20 h) increased. The impact of oxidized liposomes at different levels (1, 2, and 5%) on the salt-soluble Sawai myofibrillar proteins was also evaluated. The findings revealed that lipid oxidation products reduced the sulfhydryl content and increased the surface hydrophobicity and carbonyl content of the salt-soluble Sawai myofibrillar proteins. These results imply that the formation of yellow discoloration in Sawai muscle could be due to nonenzymatic browning reactions occurring between lipid oxidation products and amines in the muscle protein.

Keywords: amines, liposomes, Maillard reaction, microsomes, pyrroles

INTRODUCTION

Lipid oxidation of fish muscle is a major contributor to quality losses during processing and storage. Lipid oxidation causes protein denaturation, discoloration, and an off-flavor. Lipid hydroperoxides degrade during lipid oxidation, producing carbonyl molecules such as aldehydes and ketones. Aldehydes are secondary products that have drawn a lot of attention due to their unpleasant odor and affinity for amino acids. It has long been known that nonenzymatic browning occurs when lipids are oxidized in the presence of protein. The nonenzymatic browning is the result of condensation of aldehydes and amines via Schiff base reactions, which starts with the oxidation of lipids in muscle foods (Papuc et al., 2017; Wu et al., 2022). Pyrrolation is responsible for the development of brown pigments in fish muscle, which can reduce its consumer appeal (Jones, 1963). Pyrrolation is a chemical reaction that occurs in fish muscle during storage, especially in frozen or chilled conditions. It involves the formation of pyrrole compounds from amino acids and lipid oxidation products. Pyrrolation can also take place in the muscular tissue of fish during the processing stage. This process has the potential to change the coloration of fish muscle through the formation of brown or red pigments, which subsequently diminish the freshness and overall quality of the product (Ochiai and Ozawa, 2020).

The production and consumption of frozen catfish, such as Sawai (*Pangasianodon hypophthalmus*), is increasing globally. Although frozen storage significantly delays microbial deterioration, several chemical reactions can still take place during storage. One such reaction is the formation of yellow pigments (Sriket et al., 2019), which can lead to rancid odors (Santos-Yap, 1996). Changes in texture and

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muscle discoloration during storage have been reported in some fish species, including Indian catfish fillets (*P. hypophthalmus*) (Kunnath et al., 2015), basa (*Pangasius bocourti*) (Sriket and La-ongnual, 2018), Sawai (*P. hypophthalmus*) (Sriket et al., 2019), and giant catfish (*Pangasianodon gigas*) (Chaijan et al., 2010). These changes occur because of lipid oxidation, as reported by Hematyar et al. (2019) and Singh et al. (2022).

Sawai muscle has a high lipid content (40.96% dry basis), as reported by Sriket et al. (2017), and the lipids contain a high amount of unsaturated fatty acids (62.02% of total lipid), making them prone to oxidation and muscle discoloration. Previous studies have described methods to solve this problem in other fish species, such as the use of ergothioneine-rich mushroom extracts on salmon fillets (Pahila et al., 2017) and lactic acid bacteria for controlling discoloration in tuna (Jo et al., 2023). The rejection of frozen fish products due to quality deterioration caused by discoloration makes it crucial to obtain a better understanding of the processes involved in the development of yellow pigments in Sawai protein and lipid fractions. This study aims to analyze the impact of lipid oxidation on the development of yellow pigments in the microsome and liposome systems of Sawai cultured in Thailand. The knowledge gained from this research may help food processors to develop prevention methods for this phenomenon.

MATERIALS AND METHODS

Chemicals

The chemicals used in this study were purchased from Sigma Chemical Co. and Merck and were of analytical grade. The chemicals included ferric (III) chloride, butylated hydroxytoluene (BHT), L-ascorbic acid, thiobarbituric acid (TBA), monopotassium dihydrogen phosphate, dipotassium hydrogenphosphate, *p*-dimethylamino benzaldehyde (DMAB), 2,4,6-trinitrobenzenesulfonic acid (TNBS), Triton X-100, sodium dodecyl sulfate (SDS), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), ethylenediaminetetraacetic acid (EDTA), hydrochloric acid, methanol, and chloroform.

Sample preparation

Sawai (*P. hypophthalmus*) weighing 2∼3 kg were obtained from a fish farm in Ubon Ratchathani, Thailand. The fish were sacrificed via ice-shocking, and then transported on ice (1:2, w/w) in an insulated box during transport to the laboratory. The temperature of the fish during transportation was 0∼2°C. The temperature was monitored using a thermocouple to ensure that the temperature did not fluctuate during transportation. The transportation time was within 1 h of the fish being purchased. After arrival, the fish were cleaned with tap water, filleted, deskinned, and sliced to 1∼2 cm thicknesses. The resulting slices were minced to achieve a uniform mixture.

Preparation of Sawai microsomal fraction, liposomes, and salt-soluble myofibrillar proteins (SSP)

The method of Brannan and Decker (2001) with slight modifications was used to isolate Sawai muscle microsomes. In brief, 25 g of minced fish was added to 100 mL of extraction buffer (25 mM phosphate buffer/0.12 M KCl, pH 7.2), and homogenization was performed at a speed of 20,000 rpm for 2 min using a tissuemizer (Tekmar Co.). Then, the mixture was centrifuged at 10,000 *g* and 4°C for 30 min using a Sorvall Superspeed RC2-B centrifuge (Thermo Fisher Scientific, Inc.). The collected supernatant was subjected to ultracentrifugation at 100,000 *g* for 60 min using a Sorvall Ultra 80 (DuPont) to obtain a pellet containing the insoluble muscle components, including the microsomes. The myofibrillar proteins were collected from the pellet and solubilized in extraction buffer. The microsome-containing pellet was isolated by ultracentrifugation at 100,000 *g* for 60 min. The method described by Lowry et al. (1951) was used to calculate the protein content of the microsomal fraction. The obtained microsomes were adjusted to 30 mg of protein/mL using extraction buffer and stored at −60°C until further use.

To extract the lipids from the Sawai microsomes, one part of microsome was homogenized for 2 min with five parts of solvent (methanol/chloroform, 1:2). The solvent phase was collected and evaporated under nitrogen. Next, liposomes were prepared from the isolated Sawai phospholipids using the method described by Decker and Hultin (1990). The fish microsome lipid (5 mg/mL) was dispersed in extraction buffer using a Potter-Elvehjem homogenizer and then sonicated in an ice bathtub for 30 min using a sonic dismembrator (Model 500, Thermo Fisher Scientific, Inc.) at 35% amplitude with 5-s repeating cycles.

SSP were isolated using a modified method described by Thanonkaew et al. (2006). The natural actomyosin (NAM) pellet was mixed with 30% glycerol (v/v) and kept at −60°C. Before analysis, the frozen NAM was defrosted using flowing tap water. Chilled water (10 volume) was added to the thawed NAM to remove the glycerol and gently stirred for 30 min at 4°C. After centrifugation at 8,000 *g* and 4°C for 25 min, the resultant NAM mixture was stored on ice for further use. The concentration of protein in the NAM was measured using a biuret method, as described by Robinson and Hogden (1940).

During the lipid oxidation of liposomes and the microsomal fraction of Sawai muscle, to accelerate the oxidation of lipids in the microsomal systems or liposomes, a nonenzymatic iron redox cycling system was used fol-

lowing the process of Thanonkaew et al. (2006) with a few adjustments. The reaction mixture consisted of 200 μM ascorbate and 5 mg of lipid (for liposomes) or 5 mg of fish microsomal protein per mL of extraction buffer with varying concentrations of $FeCl_3$ (25, 50, and 100 μM). The assay medium was sampled at different intervals and tested for thiobarbituric acid-reactive substances (TBARS), free amines, color, and pyrrolization, as explained below.

To evaluate the effect of lipid oxidation on the chemical properties of SSP and NAM, solutions with the highest levels of these products were added directly to the SSP at varying concentrations (1, 2, and 5%). A control group was created by using an equivalent quantity of water. Each sample was then placed in an agitating incubator (INOVATM 4080, New Brunswick Scientific Co., Inc.) and incubated under atmospheric conditions at 37°C for a duration of 9 h. The samples were subsequently analyzed for sulfhydryl content, surface hydrophobicity (SoANS), and carbonyl content using the methods outlined below.

Measurement of physical and chemical alterations in the SSP, microsomes, and liposomes

To assess the level of lipid oxidation, the TBARS level was measured using a modified method based on the procedure developed by Mcdonald and Hultin (1987). The TBA stock mixture was composed of 0.375% TBA and 15% trichloroacetic acid (w/v) in 0.25 M HCl. First, 3 mL of 2% BHT in ethanol was added to 100 mL of the TBA stock solution. Then, microsomes were mixed with TBA solution at a ratio of 1:2 and vortexed. The mixture was then kept at 95°C and cooled to ambient temperature after 15 min. Next, the mixture was centrifuged at 2,000 *g* for 15 min. The optical density of the supernatant was read at 532 nm, and the results were reported as μM of TBARS per mg of microsomal protein. The concentrations of TBARS were measured based on a standard curve of malonaldehyde plotted using 1,1,3,3-tetraethoxypropane.

Color values

The color of the microsomes and liposomes was evaluated using a colorimeter (Model ColorFlex EZ, HunterLab), and the readings were noted using the CIE-$L^*a^*b^*$ color system.

Free amine groups

The spectroscopic method of Kubo and Mori (2005), with minimal modifications, was used to determine the free amine groups. The sample was diluted four times with 5% Triton X-100 and kept for 25 min at ambient temperature. Next, 100 mM TNBS (30 μL) was mixed in a diluted solution (1.5 mL). The mixture was stored for 60 min at ambient temperature, and the development of the resulting derivatives of trinitrophenyl was observed spectrophotometrically at 420 nm using a UV-visible spectrophotometer (UV-210PC, Shimadzu Scientific Instruments). A blank was produced similarly, using buffer in place of the liposomes or microsomes. Concentrations were determined based on the standard curve made with glycine.

Formation of pyrrole compounds

To assess nonenzymatic browning, phospholipid pyrrolization was used as an index, following the technique of Hidalgo et al. (2004) with minor changes. An equal amount (1:1) of 25 mM phosphate buffer containing 3% SDS was added to the sample for dilution. The resulting mixture (1 mL) was added to 0.134 M Ehrlich reagent (160 μL). The Ehrlich reagent was created by adding p-DMAB (200 mg) to ethanol (2 mL), and then making the volume up to 8 mL using 1.25 N HCl. The mixture was kept for 30 min at 45°C, and the optical density was read at 570 nm. To remove the protein following color formation, microsome samples were centrifuged at 1,600 *g* for 30 min. A blank was made similarly, using buffer in place of the liposomes or microsome.

Total sulfhydryl content

The total sulfhydryl content of the SSP was analyzed using DTNB based on the techniques established by Thanonkaew et al. (2006), with some slight modifications. Specifically, 1 mL of a 5 mg/mL SSP solution was combined with 9 mL of Tris-HCl buffer (0.2 M, pH 6.8), containing EDTA (10 mM), SDS (2%), and urea (8 M). One milliliter of this mixture was added to 100 μL of 0.1% DTNB, which was then incubated for 30 min at 40°C. The optical density was read at 412 nm using KCl (0.6 M) solution as the blank, and the sulfhydryl content was evaluated using an extinction coefficient of 13,500 $M^{-1}cm^{-1}$.

SoANS

The SoANS (surface hydrophobicity measured by anilinonaphthalene-sulfonic acid) of the SSP was determined using ANS (8-anilino-1-naphthalenesulfonic acid) as a probe, based on the approach developed by Benjakul et al. (1997). First, 10 mM sodium phosphate buffer (pH 6.0) with 0.6 M NaCl was used to dilute the SSP solution to obtain final protein concentrations of 1, 0.5, 0.25, and 0.125 mg/mL. The resulting protein solutions were then incubated at ambient temperature for 15 min. A spectrofluorometer (RF-15001, Shimadzu Scientific Instruments) with an emission wavelength of 485 nm and an excitation wavelength of 374 nm was then used to measure the fluorescence intensity of the ANS-conjugates that were created after mixing diluted protein solution (2 mL) with 20 μL of 8 mM ANS in 0.1 M phosphate buffer (pH 7.0). The SoANS value was calculated as the initial slope of the

fluorescence intensity versus the protein concentration.

Carbonyl content

To determine the carbonyl content of SSP, DNPH (2,4-dinitrophenyl hydrazine) derivatization was used, following the protocol of Levine et al. (1994) and Larsson and Undeland (2010), with some adjustments. A homogenate of 0.5 g of SSP in 10 mL of buffer (50 mM Tris, 1 mM EDTA, pH 7.4) containing 0.01% BHT was prepared using an Ultra Turrax homogenizer (IKA Labortechnik). The homogenate (300 μL) was then mixed with 30% TCA (0.5 mL) and centrifuged for 3 min at 13,400 *g* at 4°C. The resulting pellet was then incubated with 10 mM DNPH melted in 2 M HCl for 60 min. A blank was created using 2 M HCl as a substitute for the DNPH solution. The remaining DNPH was removed by washing with 1.0 mL of ethanol-ethyl acetate (1:1) and 10 mM HCl after precipitation with 0.5 mL of TCA (30%). Finally, 1 mL of 6 M guanidine chloride in 20 mM KH_2PO_4 (pH 2.3) was used to dissolve the protein pellets, which were then stored overnight at 4°C. The carbonyl content (nmol/mg protein) was calculated using the optical densities of the samples at 370 and 280 nm and an absorption coefficient at 370 nm of 22,000 $M^{-1}cm^{-1}$ for the generated hydrazones.

Statistical analysis

The experiments were conducted in triplicate, and the information was analyzed using analysis of variance. Mean judgments were performed using Duncan's multiple range test, as described by Steel and Torrie (1980). Statistical analysis was carried out using the IBM SPSS 28.0 for Windows (IBM Corp.).

RESULTS AND DISCUSSION

Modifications in TBARS of Sawai liposome and microsome

We investigated the variations in the TBARS of Sawai liposomes and microsomes during incubation at 37°C for 20 h in the presence of ascorbic acid (200 μM) and different concentrations of $FeCl_3$ (0∼100 μM). An upsurge in lipid oxidation was observed in both the liposomes (Fig. 1A) and microsomes (Fig. 1B) as the concentration of iron and reaction time increased. Ascorbic acid has the ability to reduce iron to its ferric state, resulting in the prooxidative ferrous state, which accelerates lipid oxidation via a Fenton type reaction. The lipid oxidation of Sawai liposomes and microsomes in the presence of 100 μM $FeCl_3$ was approximately 6-fold larger than that of the control samples.

Thanonkaew et al. (2006) reported that the TBARS levels in squid liposomes and microsomes increased with rising temperatures and reaction times. Chan et al. (1997) created a model system that involved positioning oxidized liposomes in a dialysis bag to investigate the connections between myoglobin and lipid oxidation products. This model was designed to prevent direct interactions between myoglobin and liposomes but to allow low molecular weight lipid oxidation products to modify myoglobin by passing through the dialysis membrane. In the present study, a similar model could have been used to determine if the products of fatty acid breakdown interact with the proteins in muscle to produce yellow pigments. Fish products that contain highly unsaturated fatty acids are susceptible to oxidization and the production of aldehydes during storage, which causes discoloration (Singh et al., 2022) via reactions with amine groups to form non-enzymatic browning products (Thanonkaew et al., 2006).

Changes in color

Fig. 2 and 3 illustrate the color changes in Sawai lipo-

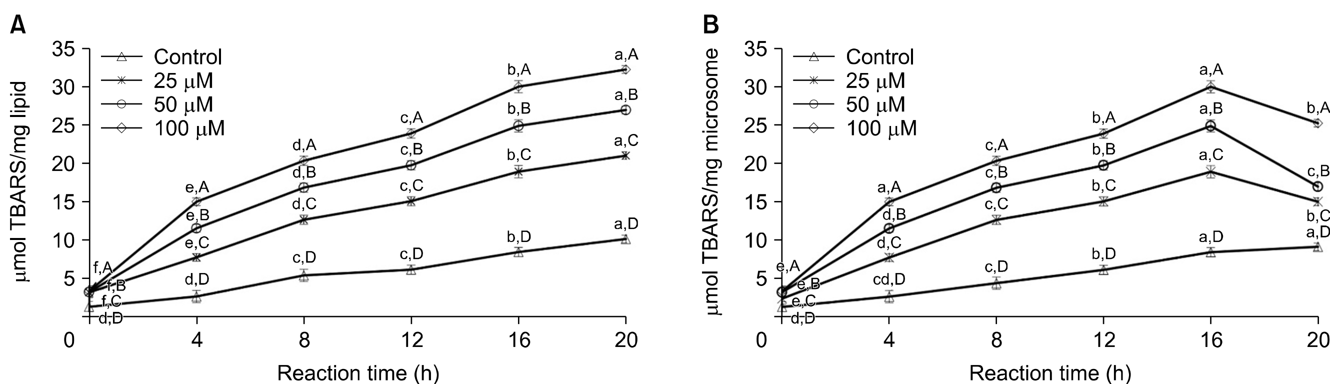
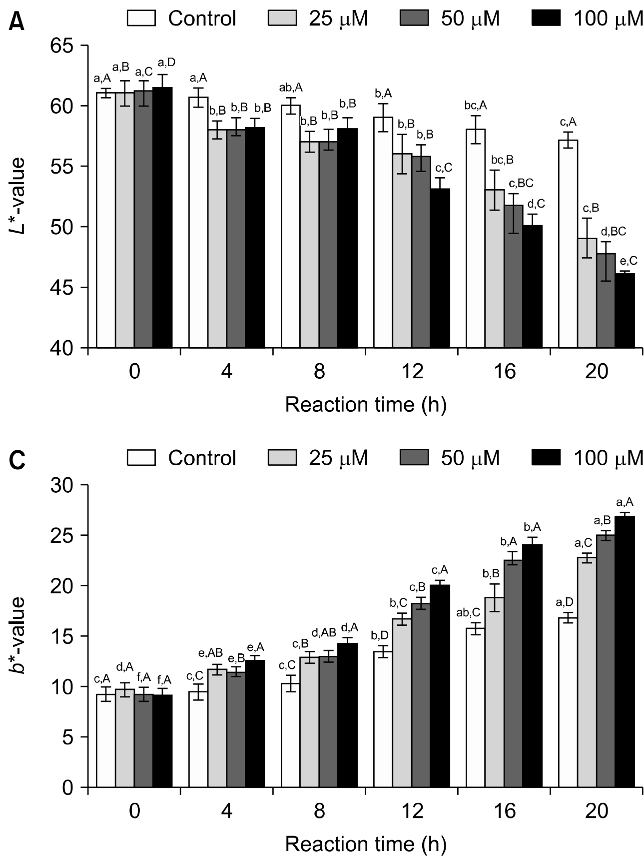


Fig. 1. Formation of thiobarbituric acid-reactive substances (TBARS) in Sawai liposome made from Sawai lipids (5 mg lipid/mL) (A) and muscle microsomes (5 mg microsomal protein/mL) (B) in the presence of 200 μM ascorbic acid and various concentrations of $FeCl_3$ (0~100 μM) during incubation at 37°C for 20 h. Data are presented as mean±standard deviation (n=3). Different lowercase letters on the line graph within the same concentration of $FeCl_3$ denote significant differences (P<0.05). Different uppercase letters on the line graph within the same reaction time denote significant differences (P<0.05).



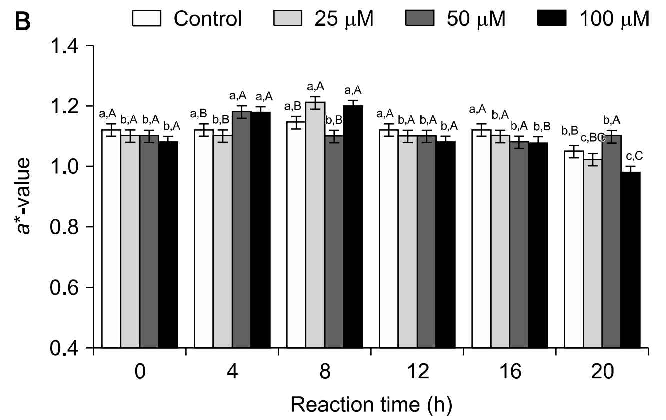
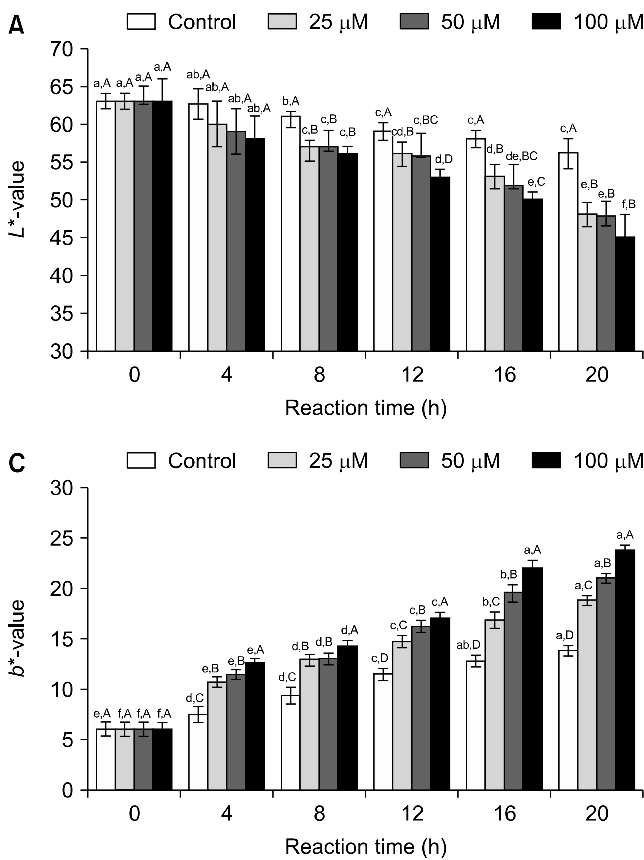


Fig. 2. Changes in L^* (A), a^* (B), and b^* (C)-value in liposomes made from Sawai lipids (5 mg lipid/mL) in the presence of 200 μM ascorbic acid and various concentrations of $FeCl_3$ (0~100 μM) during incubation at 37°C for 20 h. Data are presented as mean±standard deviation (n=3). Different lowercase letters on the bars within the same concentrations of $FeCl_3$ denote significant differences ($P<0.05$). Different uppercase letters on the bars within the same reaction time denote significant differences ($P<0.05$).



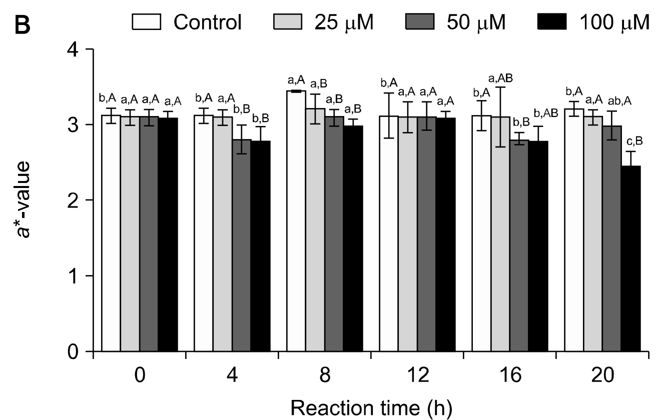


Fig. 3. Changes in L^* (A), a^* (B), and b^* (C)-value in Sawai muscle microsomes (5 mg microsomal protein/mL) in the presence of 200 μM ascorbic acid and various concentrations of $FeCl_3$ (0~100 μM) during incubation at 37°C for 20 h. Data are presented as mean±standard deviation (n=3). Different lowercase letters on the bars within the same concentrations of $FeCl_3$ denote significant differences ($P<0.05$). Different uppercase letters on the bars within the same reaction time denote significant differences ($P<0.05$).

somes and microsomes, respectively, after incubation at 37°C for 20 h in the presence of 200 μM ascorbic acid and varying concentrations of $FeCl_3$ (0～100 μM). The *L**, *a**, and *b** values of the Sawai liposomes were not significantly different from those of the control sample ($P>0.05$). However, a reduction in *L** value was detected in all samples with increasing reaction time ($P<0.05$) (Fig. 2A). No significant variations in the *a** values of the samples were detected during incubation ($P>0.05$) (Fig. 2B). The *b** values of all samples increased with reaction time ($P<0.05$). In particular, the liposomes with added iron exhibited higher *b** values compared to the control sample (Fig. 2C). A positive correlation between lipid oxidation (TBARS) (Fig. 1A) and yellow color (*b**) was observed in iron-containing liposomes. Similar color changes were observed in the Sawai microsomes when compared with the Sawai liposomes during incubation at 37°C for 20 h in the occurrence of 200 μM ascorbic acid and varying concentrations of $FeCl_3$ (0～100 μM) (Fig. 3).

The results suggest that the development of yellow pigments in Sawai can possibly be attributed to nonenzymatic browning reactions among phospholipid head groups and fatty acid decomposition products. Alternatively, browning could happen due to connections between the amines in proteins and fatty acid decomposition products (Thanonkaew et al., 2006). Aldehydes and carbonyl compounds produced by the oxidation of unsaturated fatty acids, such as heptanal, hexanal, and octanal, can react with free amino groups in proteins, causing discoloration and foul smells (Herrera and Calkins, 2022; Martin et al., 2023). Yellow discoloration associated with lipid oxidation has also been reported in squid liposomes and microsomes (Thanonkaew et al., 2006). Furthermore, lipid oxidation and yellow discoloration were more pronounced in both Sawai liposomes and microsomes with higher concentrations of iron and reaction time. This is likely due to the fact that iron is a critical catalyst for lipid oxidation and browning in muscle food.

Changes in amine groups and pyrrole compounds

Fig. 4 and 5 depict the changes in amine groups and pyrrole compounds of Sawai liposomes and microsomes during 20-h incubation at 37°C with 200 μM ascorbic acid and various concentrations of $FeCl_3$ (0～100 μM). The loss of amine groups, which is an indicator of interactions between phospholipids and lipid oxidation products, was observed in both Sawai liposomes (Fig. 4A) and microsomes (Fig. 5A) in the presence of ascorbic acid and iron. The loss of free amines occurred over the same period of time in both Sawai liposomes and microsomes, indicating that oxidative products of lipids possibly took part in the development of yellow pigments in the Sawai muscle.

Both Sawai liposomes (Fig. 4B) and microsomes (Fig. 5B) showed a significant increase in pyrroles ($P<0.05$) when treated with ascorbic acid and varying concentrations of iron. The control samples of both liposomes and microsomes exhibited a slow increase in pyrroles, particularly after 8 h of reaction time, indicating that time and temperature are significant factors in lipid oxidation. The formation of pyrroles resulting from the reactions between oxidized lipids and proteins is a crucial precursor to browning, as noted in previous studies (Li et al., 2020). Pyrrolation is the process of adding pyrrole rings to proteins, which can lead to the formation of brown pigments. The brown pigments are formed by reactions between pyrroles and amino acids, such as lysine and arginine, in the proteins. The reaction between pyrroles and amino acids is known as the Maillard reaction. The exact chemical structure of the brown pigments formed during the Maillard reaction is not well-defined because it depends on the specific amino acid, amine, and lipid oxidation products involved in the reaction (Murata, 2021).

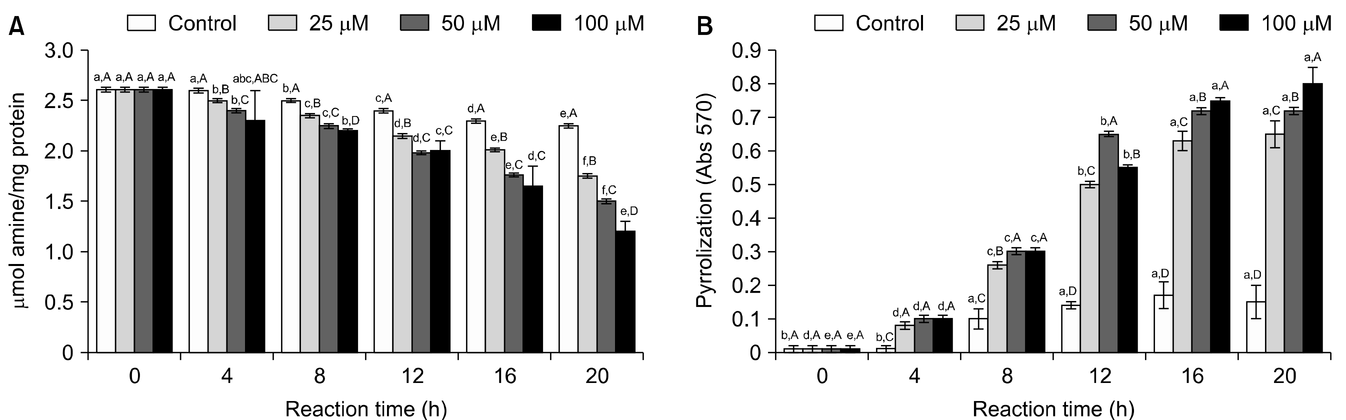


Fig. 4. Changes in amine groups (A) and the formation of pyrrole compounds (B) in liposomes made from Sawai lipids (5 mg lipid/mL) in the presence of 200 μM ascorbic acid and various concentrations of $FeCl_3$ (0~100 μM) during incubation at 37°C for 20 h. Data are presented as mean±standard deviation (n=3). Different lowercase letters on the bars within the same concentrations of $FeCl_3$ denote significant differences ($P<0.05$). Different uppercase letters on the bars within the same reaction time denote significant differences ($P<0.05$).

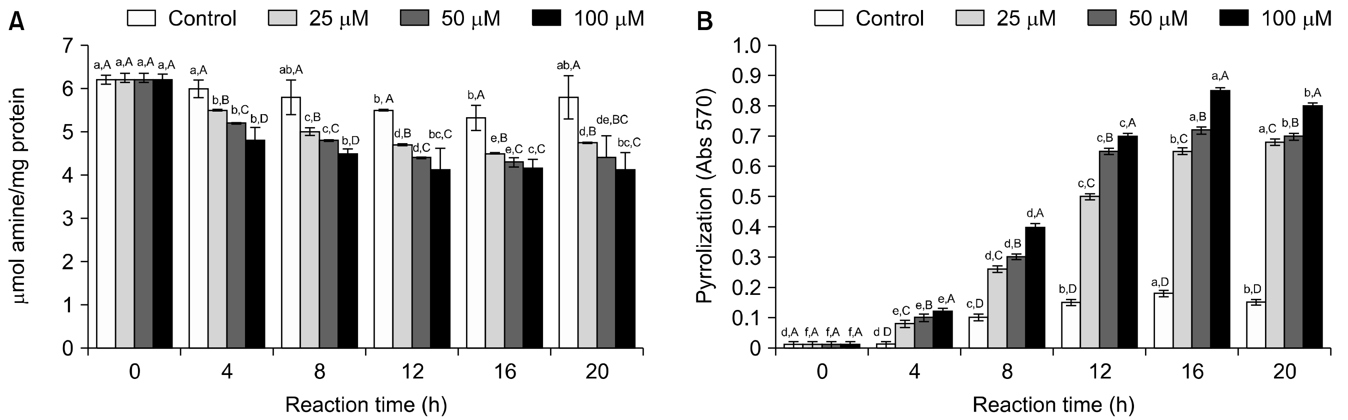


Fig. 5. Changes in amine groups (A) and the formation of pyrrole compounds (B) in Sawai muscle microsomes (5 mg microsomal protein/mL) in the presence of 200 μM ascorbic acid and various concentrations of $FeCl_3$ (0~100 μM) during incubation at 37°C for 20 h. Data are presented as mean±standard deviation (n=3). Different lowercase letters on the bars within the same concentrations of $FeCl_3$ denote significant differences ($P<0.05$). Different uppercase letters on the bars within the same reaction time denote significant differences ($P<0.05$).

However, the presence of some compounds, including pyrroles, furans, pyrazines, oxazoles, thiazoles, and other heterocyclic compounds, which can contribute to the formation of brown pigments in fish muscle, have been reported previously (Mottram, 1998; Tamanna and Mahmood, 2015; Murata, 2021).

In the presence of ascorbate and iron, the levels of free amines decreased, and simultaneously, the formation of pyrrole compounds, TBARS, and yellow pigments occurred. This phenomenon suggests that oxidative products of lipids interact with amines, leading to the formation of yellow pigments (Thanonkaew et al., 2006).

Changes in Sawai SSP due to oxidized Sawai liposomes

To investigate the effects of oxidative products of lipids from Sawai liposomes on the characteristics of Sawai SSP, oxidized Sawai liposomes were directly added to SSP at various concentrations (1, 2, and 5%) and developed at 37°C for 9 h. The results of this experiment showed that the sulfhydryl content of SSP decreased (Fig. 6A) with in-

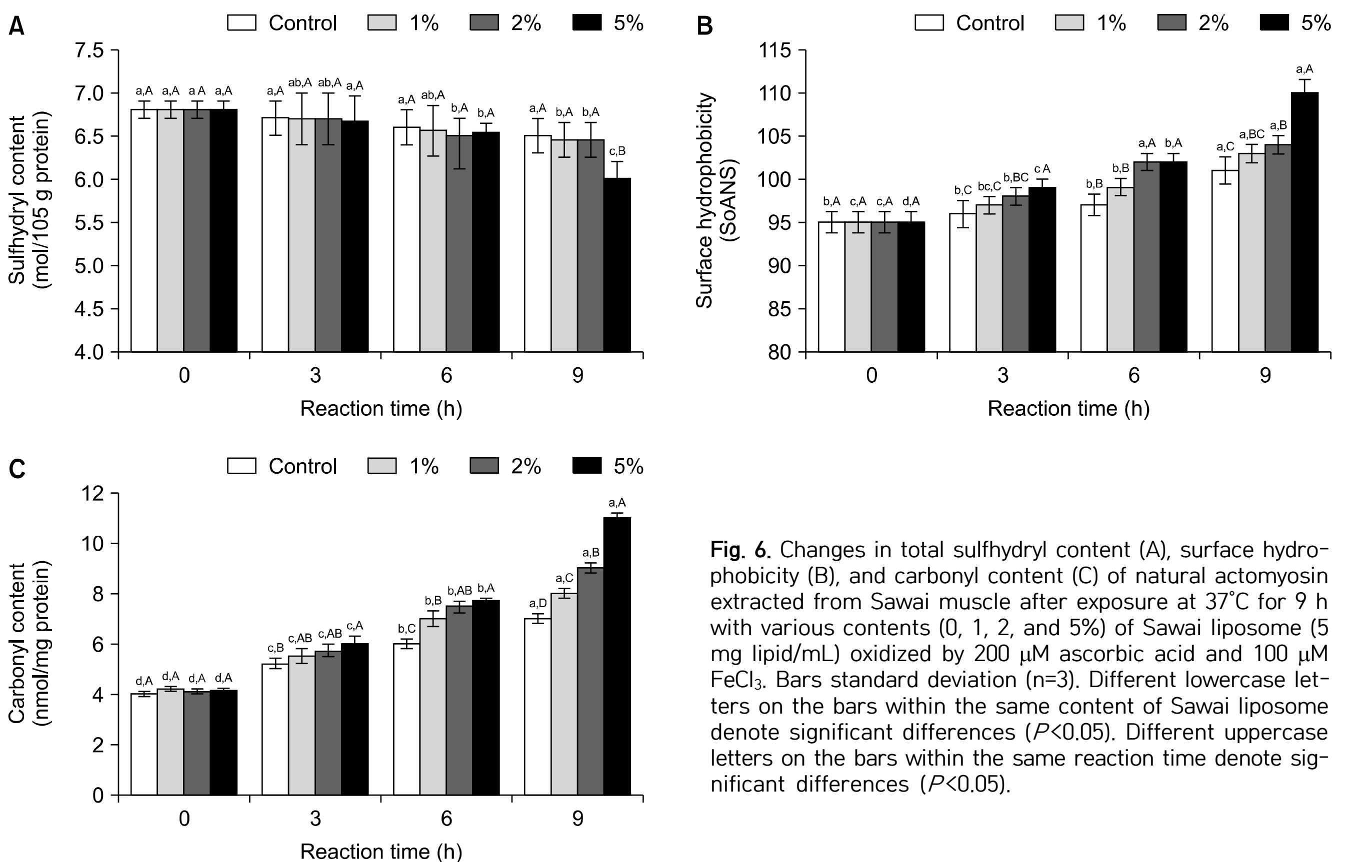


Fig. 6. Changes in total sulfhydryl content (A), surface hydrophobicity (B), and carbonyl content (C) of natural actomyosin extracted from Sawai muscle after exposure at 37°C for 9 h with various contents (0, 1, 2, and 5%) of Sawai liposome (5 mg lipid/mL) oxidized by 200 μM ascorbic acid and 100 μM $FeCl_3$. Bars standard deviation (n=3). Different lowercase letters on the bars within the same content of Sawai liposome denote significant differences ($P<0.05$). Different uppercase letters on the bars within the same reaction time denote significant differences ($P<0.05$).

creasing development time in the presence of higher concentrations (5%) of oxidized Sawai liposomes. The SoANS of SSP increased as the concentration of oxidized Sawai liposomes increased, particularly after 9 h of incubation (Fig. 6B). Moreover, the addition of oxidized Sawai liposomes to the SSP had a significant (P<0.05) impact on the carbonyl content (Fig. 6C), which increased in a dose-dependent manner. The carbonyl content is an indicator of protein oxidation (Wen et al., 2019). Malondialdehyde, a secondary oxidative product of lipids, can covalently attach to proteins, resulting in the formation of some of the carbonyls observed. The presence of carbonyl groups and products from lipid oxidation are known to contribute to the yellow discoloration of fish muscle protein (Tongnuanchan et al., 2011). These results suggest that oxidized Sawai liposomes are capable of modifying the properties of Sawai SSP.

In conclusion, the susceptibility of Sawai lipids in liposomes and microsomes systems to lipid oxidation increased with the addition of iron and ascorbic acid, and this vulnerability was observed to increase with increasing iron concentration and reaction time. The oxidation of Sawai lipids produced yellow pigments in both liposomes and microsomes. Moreover, oxidized Sawai liposomes were found to increase protein oxidation in SSP. The findings of this research indicate that nonenzymatic browning reactions in Sawai muscle predominantly involve interactions between lipid oxidation products and the amines of proteins, suggesting a positive connection between the oxidation of lipids and the formation of yellow pigments in Sawai meat.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: UP, SB. Analysis and interpretation: PK, SN. Data collection: CS. Writing the article: CS. Critical revision of the article: UP, SB, TS. Final approval of the article: all authors. Statistical analysis: CS, TS. Obtained funding: TS. Overall responsibility: TS, SN.

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