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RESEARCH PAPER

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Effect of Iron on Physicochemical Changes of Sawai (*Pangasianodon hypophthalmus*) Pastes During Multiple Freeze-Thaw Cycles

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Abstract

Effects of different iron concentrations (0, 5, 10, 15, 20 and 25 ppm) on the lipid oxidation, color and protein changes of Sawai (*Pangasianodon hypophthalmus*) pastes subjected to multiple freeze-thaw cycles, were investigated. TBARS value of all samples increased as the number of freeze-thaw cycle increased ($P<0.05$). The increase in TBARS values of Sawai pastes induced by iron was in a dose dependent manner. Increasing in lipid oxidation of the samples containing iron was concomitant with the increase in b*-value (yellowness). The changes in protein oxidations and protein solubility were more pronounced when freeze-thaw cycles increased. However, iron at all concentrations showed negligible effects on those changes. Decreasing in Ca^{2+}-ATPase activity of fish natural actomyosin was found in all samples with increasing freeze-thawing process. SDS-PAGE showed that proteins with high MW were observed in the sample with added iron. Therefore, iron induced the yellow discoloration in Sawai muscle, associated with lipid oxidation, particularly with multiple freeze-thaw cycles.

Introduction

Catfish, such as Sawai (*Pangasianodon hypophthalmus*), have been increasingly produced and consumed due to its taste and nutritional values. Vietnam is the largest producer followed by Thailand, Cambodia, Lao People's Democratic Republic, Myanmar, Bangladesh and China (Orban *et al.,* 2008). Thailand is an important source of catfish production including Sawai, Basa (*Pangasius bocourti*) and giant catfish (*Pangasianodon gigas*). Normally, catfish have been exported as frozen fillet. Although, frozen storage can effectively prevent the microbiological deterioration, various chemical reactions still take place. During storage and freeze-thaw process, fish fillet undergoes quality deterioration (muscle discoloration or yellowness), which is sometimes accompanied by the development of a rancid odor. Texture also tends to become tougher and rubbery. Quality deterioration in frozen fish causes the rejection of the product and economic loss. Textural changes and muscle discoloration of some fish species including giant catfish (*P. gigas*) (Rawdkuen, Jongjareonrak, Benjakul, & Chaijan, 2008), herring fillets (Hamre, Lie, & Sandnes, 2003) and Indian catfish fillet (Kunnath, Lekshmi, Chouksey, Kannuchamy, & Gudipati, 2015) during storage have been reported. Those changes have been reported to result from lipid oxidation (Kunnath *et al.,* 2015; Rawdkuen *et al.,* 2008).

Several hypotheses from different studies showed that the contamination of iron in fish during handling and processing (Thanonkaew, Benjakul, Visessanguan, &

Decker, 2006) or endogenous iron (Chaijan, 2008) and the released iron from heme protein as affected by storage conditions (Maqsood, Benjakul, & Kamal-Eldin, 2012) may contribute to the acceleration of lipid oxidation of fish muscle. Iron and heme proteins have also been reported to have the capacity to promote lipid oxidation in muscle foods (Kathirvel & Richards, 2012; Min & Ahn, 2005). As a consequence, lipid oxidation products such as peroxides, aldehydes, and ketones are formed. Interaction of peroxides or carbonylic peroxide decomposition products with active groups of protein can lead to protein polymerization or aggregation as well as discoloration in fish. A preliminary study showed that Sawai meat contained high amounts of unsaturated fatty acids and it is very susceptible to oxidation. Lipid oxidation is of great concern to the food industries and consumers since it contributes to the development of poor color, odor and texture as well as reduces the nutritive values (Logan, Nienaber, & Pan, 2013; Undeland, 2016). Muscle discoloration related with lipid oxidation of some fish species has been reported. However, the information regarding lipid oxidation, discoloration and physicochemical changes in muscle proteins influenced by iron in Sawai during extended frozen storage is limited. Therefore, the objective of this research was to study the effects of iron on lipid oxidation, discoloration and physicochemical changes of Sawai muscle during multiple freeze-thaw cycles.

Materials and Methods

Chemicals

Ammonium molybdate, 5-5´-dithio-bis (2-nitrobenzoic acid) (DTNB), adenosine 5´-triphosphate (ATP), *p*-nitrophenyl-α-glucopyranoside, *p*-nitrophenyl-*N*-acetyl-β-D-glucose amide, 1-anilinonaphthalene-8-sulphonic acid (ANS) and iron (II) chloride, thiobarbituric acid (TBA) and malondialdehyde (MDA) were purchased from Sigma (St. Louis, MO, USA). Potassium chloride (KCl), sodium dodecyl sulphate (SDS) and urea were obtained from Ajax Finechem (Wellington, New Zealand).

Sample Preparation

Sawai (*P. hypophthalmus*) weighing 2-3 kg, killed by ice-shocking, were purchased from a fish farm in Ubon Ratchathani, Thailand. The fish were kept in ice using a fish/ice ratio of 1:2 (w/w) during transportation to the Program in Food Science and Technology, Faculty of Agriculture, Ubon Ratchathani Rajabhat University. Upon arrival, fish were washed with tap water, filleted, deskinned and cut into slices with a thickness of 1-2 cm. The slices were ground using a blender (Model MX-898N, Panasonic, Panasonic Sdn. Bhd., Kuala Lumpur, Malaysia) to obtain uniform material. Minced fish were frozen in liquid nitrogen and then powdered in a blender. The fish powders were mixed with 8 mL of sterilized deionized water (GenPure^{TM}, Thermo Fisher Scientific, Waltham, MA, USA) (control) or stock solution containing iron ($FeCl_2$) to obtain the final concentrations of 5, 10, 15, 20 and 25 ppm. The fish pastes were packaged in polyethylene bags and frozen at -20°C using an air blast-freezer for 48 h, followed by thawing using running tap water (25-27°C) until the core temperature reached 0-2°C. The mixtures were subjected to different freeze-thaw cycles (0, 1, 2, 3, 5 and 7 cycles).

Determination of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were determined as described by Buege and Aust (1978). Fish paste (2 g), containing various concentrations of iron, was dispersed in 10 mL of thiobarbituric acid solution (0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl). The mixture was heated in boiling water for 10 min, followed by cooling in running tap water. The mixture was centrifuged at 3600 ×g for 20 min using a refrigerated centrifuge (Model CR22N, Hitachi, Hitachi Koki Co., Ltd., Tokyo, Japan) at 25°C. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Model UV 1800, Shimadzu, Kyoto, Japan). The standard curve was prepared using malondialdehyde (Sigma), and TBARS were expressed as mg malonaldehyde/kg sample.

Determination of Color

The fish paste was spread in the sample cup and color was measured through the bottom of the sample cup using a colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA) and reported in the CIE color profile system as L*, a* and b*-values.

Determination of Surface Hydrophobicity

Surface hydrophobicity (SoANS) was determined as described by Benjakul, Seymour, Morrissey and An (1997) using ANS as a probe. Natural actomyosin (NAM) was prepared as described by Benjakul et al. (1997). Fish paste was homogenized in chilled 0.6 M KCl, pH 7.0 at a ratio of 1:10 (w/v) using a homogenizer (IKA, Labortechnik, Selangor, Malaysia). To avoid overheating, the sample was placed in ice and homogenized for 20 s, followed by a 20 s rest interval for a total extraction time of 4 min. The homogenate was centrifuged at 5000 ×g for 30 min at 4ºC using a refrigerated centrifuge. To the supernatant, three volumes of chilled deionized water (4ºC) were added to precipitate NAM. The NAM was collected by centrifuging at 5000 ×g for 20 min at 4ºC. The NAM pellet was dissolved in chilled 0.6 M KCl, pH 7.0 for 30 min at 4ºC and then centrifuged at 5000 ×g for 20 min at 4ºC. The supernatant was collected and used as NAM.

NAM solution (4 mg/mL) was diluted in 10 mM sodium phosphate buffer, pH 6.0, containing 0.6 M NaCl to produce protein concentrations of 0.125, 0.25, 0.5 and 1 mg/mL, followed by incubation at room temperature (25-28ºC) for 10 min. The diluted protein solution (2 mL) was mixed with 20 µL of 8 mM ANS in 0.1 M phosphate buffer, pH 7.0 and the fluorescence intensity of ANS-conjugates was immediately measured using a spectrofluorometer (RF-15001, Shimadzu, Kyoto, Japan) at an excitation wavelength of 374 nm and an emission wavelength of 485 nm. The initial slope of the plot of fluorescence intensity versus protein concentration was referred to as SoANS.

Determination of Total Sulfhydryl Content

The total sulfhydryl content was determined using DTNB according to the method of Ellman (1959) as modified by Benjakul et al. (1997). One mL of NAM solution (4 mg protein/mL determined using the Biuret method (Robinson & Hogden, 1940) with bovine serum albumin (Sigma) as the standard assuming 100% purity) was mixed with 9 mL of 0.2 M Tris-HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM EDTA. Four mL of the mixture were mixed with 0.4 mL of 0.1% DTNB and incubated at 40°C for 25 min. The absorbance at 412 nm was measured using the spectrophotometer and a 0.6 M KCl solution was used as a blank. The sulfhydryl content was calculated using the extinction coefficient of 13500 M^{-1} cm^{-1} (Ellman, 1959).

Determination of Disulfide Bond Content

The disulfide bond content was determined using the NTSB assay according to the method of Thannhauser, Konishi and Scheraga (1987). To 0.5 mL of NAM solution (4 mg/mL), 3.0 mL of freshly prepared NTSB assay solution were added. The mixture was mixed thoroughly and incubated in the dark at room temperature for 25 min. The absorbance at 412 nm was measured using the spectrophotometer. The disulfide bond content was calculated using the extinction coefficient of 13900 M^{-1} cm^{-1} (Ellman, 1959).

Determination of Ca^{2+}-ATPase Activity

ATPase activity was determined using the method of Benjakul et al. (1997). NAM was diluted to 3–5 mg/mL with 0.6 M KCl, pH 7.0. Diluted NAM solution (0.5 mL) was added to 0.3 mL of 0.5 M Tris–maleate, pH 7.0. Then, 0.5 mL of 10 mM $CaCl_2$ and 3.45 mL of distilled water were added. To initiate the reaction, 0.25 mL of 20 mM ATP were added. The reaction was done for 10 min at 25ºC and terminated by adding 2.5 mL of chilled (4ºC) 15% (w/v) trichloroacetic acid. The reaction mixture was centrifuged at 3500 ×g for 5 min and the inorganic phosphate liberated in the supernatant was measured using the method of Fiske and Subbarow (1925). Briefly, the supernatant (250 µL) was mixed with deionized water (625 µL), Elon reagent (1,250 µL) and ammonium molybdate (250 µL). The mixture was incubated at 25ºC for 45 min. Inorganic phosphate was measured by reading the absorbance at 640 nm. KH_2PO_4 was used as phosphate standard. Specific activity was expressed as µmol inorganic phosphate (Pi) released/mg protein/min. A blank solution was prepared by adding chilled trichloroacetic acid prior to addition of ATP.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was used to monitor the polymerization of the modified proteins. The fish paste was solubilized in 5% SDS (1:9, w/v) and dissolved in sample buffer with and without β-mercaptoethanol. SDS-PAGE was done using 4% stacking gels and 10% running gels (Laemmli, 1970). Proteins (15 µg) determined using the Biuret method were loaded onto the gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini-Protean II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid for 3 hr and destained with 50% (v/v) methanol and 7.5% (v/v) acetic acid for 15 min, followed by 5% (v/v) methanol and 7.5% (v/v) acetic acid for 3 hr. High molecular weight markers (GE healthcare UK Limited, Buckinghamshire, UK) were used for estimation of MW of protein bands.

Determination of Protein Solubility

Solubility was determined according to Benjakul and Bauer (2000). To 1 g fish paste, 20 mL of 0.6 M KCl were added and the mixture was homogenized for 1 min at a speed of 12000 rpm, using an IKA homogenizer. The homogenate was stirred at 4°C for 4 h, followed by centrifuging at 8500 ×g for 30 min at 4°C. To 10 mL of supernatant, cold (4°C) 50% (w/v) trichloroacetic acid was added to obtain a final concentration of 10%. The precipitate was washed with 10% trichloroacetic acid and solubilized in 0.5 M NaOH. The fish paste was also directly solubilized using 0.5 M NaOH to determine total protein. Protein content was determined and expressed as the percentage of total protein in the sample.

Statistical Analysis

Experiments were run in triplicate. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) (Steel & Torrie, 1980). Statistical analyses were done using the Statistical Package for Social Science (SPSS 13.0 for Windows, SPSS Inc., Chicago, IL, USA). The significance level was set at $P<0.05$.

Results and Discussion

Changes in TBARS Values

Changes in TBARS in Sawai paste samples with and without iron are shown in Figure 1. The differences in TBARS values found in Sawai pastes with and without iron addition suggested that iron addition increased TBARS after sample mixing. The catalytic effect of iron and heme protein on lipid oxidation has been reported (Maqsood *et al.,* 2012).

The increase in TBARS for all samples with increasing freeze-thaw cycles was significant (P<0.05), with the control sample's TBARS increasing sharply after 2 cycles (P<0.05). The repeated ice crystal formation during multiple freeze-thaw cycles could disrupt the muscle structure of the samples (Benjakul *et al.,* 2000). Disruption of muscle food structure induced by multiple freeze-thaw cycles has been reported (Ali, Rajput, Li, Zhang, & Zhou, 2016). The freeze–thawing process could affect the physical organization of membrane lipids, which could impact lipid oxidation pathways. As a consequence, the formation of some compounds such as aldehydes and hydrocarbons caused by the lipid oxidation could interact with protein to produce off-colors (Wasowicz, Gramza, Hes, Jeleñ, Korczak, & Malecka, 2004). For each freeze-thaw cycle, samples with iron had higher TBARS values compared to control (P<0.05). In addition, the rate of lipid oxidation induced by iron was in a dose dependent manner.

Changes in Colors

Changes in L*, a* and b* values are shown in Figure 2. After iron addition, there was no difference in L* (lightness), a* (redness) and b* (yellowness) values of the Sawai pastes, compared to the control (P≥0.05). However, the decrease in L* values of all samples was observed when the number of freeze-thaw cycles increased (Figure 2a). It was noted that initially, iron added samples had lower L* value than control (P<0.05).

The decrease in a* value in all samples was observed when the number of freeze-thawing cycles increased (P<0.05) (Figure 2b). For each freeze-thaw cycle, there was no difference in a* values between samples with iron and controls (Figure 2b). The decreased a* value in all samples might result from a water bleaching effect. Jeong et al. (2011) reported that a* value and heme pigment content of beef muscle decreased as the number of freeze-thawings increased. Pigment degradation can also lead to changes in muscle color (Alonso, Muela, Tenas, Calanche, Roncales, & Beltran, 2016). Freeze-thawing effect on drip loss and a* value of muscle has been reported (Alonso *et al.,* 2016; Cheng, Sun, & Pu, 2016).

The increase in b* values of all samples increased with freeze-thawing up to 5 cycles (P<0.05). However, higher b* values for each cycle with iron compared to the controls were found (Figure 2c). A positive correlation (R^2=0.97) between lipid oxidation (TBARS) and yellow color (b*) formation was found in the samples containing iron. In addition, at the first two freeze-thaw cycles, the gradual increase in b* value of controls was correlated (R^2=0.97) with the slow increase in TBARS value (Figure 1). Aldehydes, ketones and carbonyl compound products from the oxidation of unsaturated fatty acids can react with free amino groups in protein. These reactions lead to the formation of discoloration and production of unpleasant odors (Pokorny, 1981; Wsowicz, Gramza, Hes, Jeleñ, Korczak Malecka, 2004). Fish quality deterioration such as lipid oxidation and discoloration induced by freeze-thawing has been reported (Mousakhani-Ganjeh, Hamdami, & Soltanizadeh, 2015; Mousakhani-Ganjeh, Hamdami, & Soltanizadeh, 2016). These results indicated that iron addition can facilitate the yellow color formation of Sawai pastes, particularly with increasing numbers of freeze-thaw cycles.

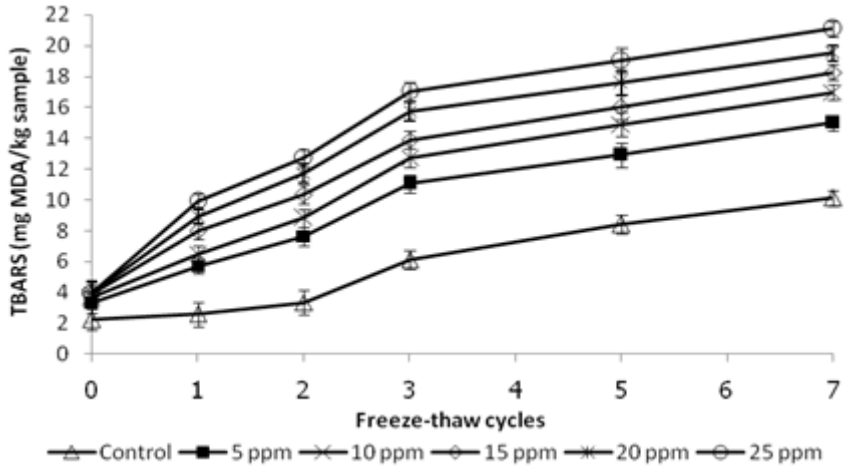

Figure 1. Changes in TBARS values of Sawai paste with different concentrations of iron during multiple freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

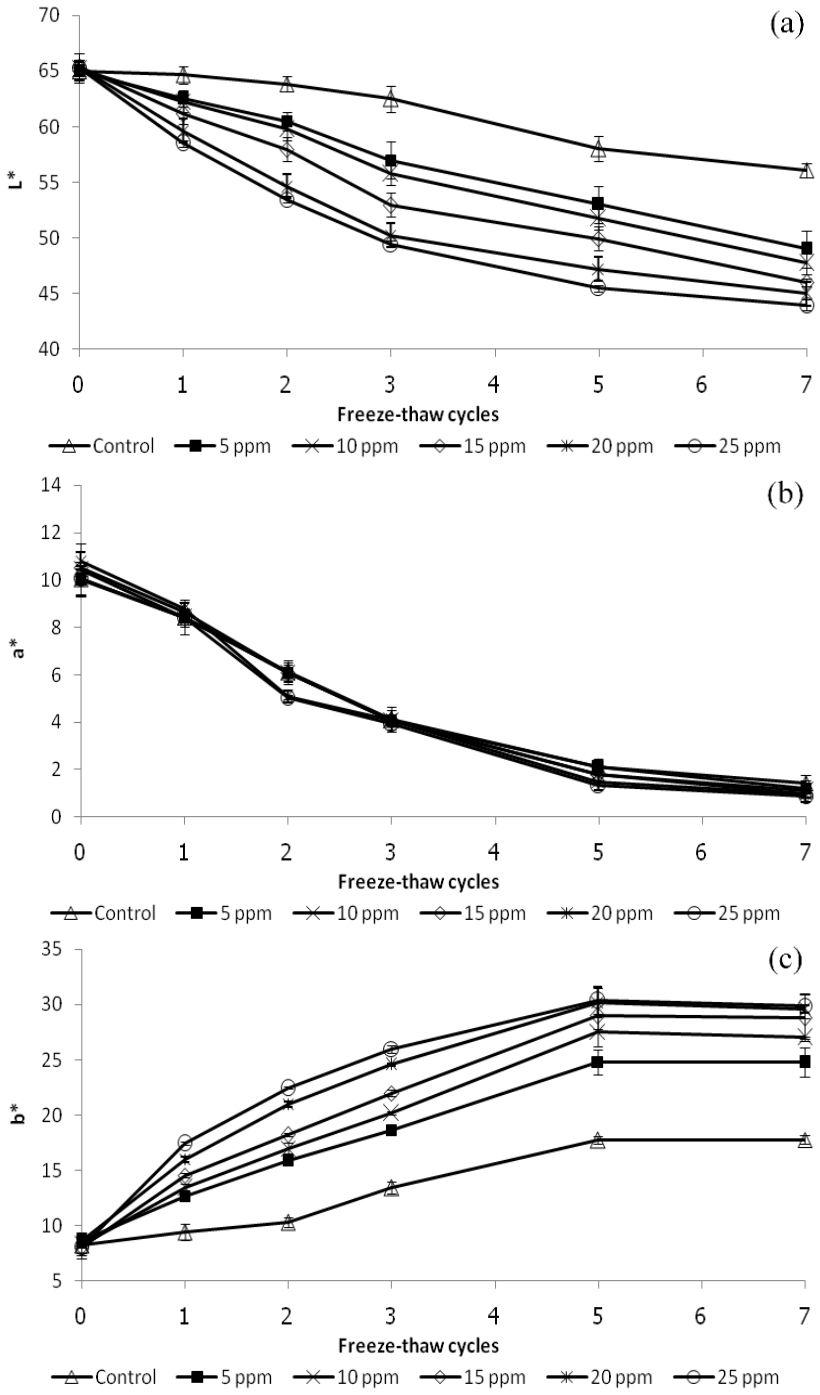

Figure 2. Changes in L* value (a) a* value (b) and b* value (c) of Sawai paste with different concentrations of iron during multiple freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

Changes in Surface Hydrophobicity

Changes in surface hydrophobicity (SoANS) of natural actomyosin extracted from Sawai pastes are shown in Figure 3a. SoANS gradually increased as the number of freeze-thaw cycles increased, regardless of iron concentration (Figure 3a). However, after 5 freeze-thaw cycles, SoANS of all samples remained unchanged ($P \geq 0.05$). The hydrophilic residues are generally exposed to water, while the hydrophobic groups are localized in the molecule. Sriket et al. (2007) reported an increase in SoANS of NAM extracted from black tiger and white shrimps during 5 freeze-thaw cycles. The increase in surface hydrophobicity of freeze-thawed Sawai pastes was in agreement with other studies that increased surface hydrophobicity was observed as frozen storage time increased (Kobayashi & Park, 2017; Li, Kong, Xia, Liu, & Li, 2013). In addition, some studies also showed that the increased protein oxidation leading to increased hydrophobicity correlated with increased lipid

oxidation (Kong, Guo, Xia, Liu, Li, & Chen, 2013; Xia, Kong, Liu, & Liu, 2009), suggesting that protein structural changes might result from the reaction between functional groups of proteins and oxidation products of polyunsaturated fatty acids (Tokur & Korkmaz, 2007). However, in these experiments, an increase in SoANS was more pronounced when the number of freeze-thaw cycles increased, while the addition of iron had a negligible effect on the SoANS.

Changes in Sulfhydryl and Disulfide Bond Contents

Changes in sulfhydryl content and disulfide bond content of Sawai NAM are shown in Figure 3. Decreasing sulfhydryl content for all samples was observed with increasing freeze-thaw cycles ($P<0.05$) (Figure 3b). For the first three freeze-thaw cycles, different sulfhydryl contents for all samples were not observed ($P\geq0.05$). However, after three freeze-thaw cycles, samples with iron (5-25 ppm) had slightly lower sulfhydryl content, compared to the control. These findings were slightly different from Thanonkaew et al. (2006) who reported that there is no difference in sulfhydryl content between samples with iron and controls. This might be due to the different specificity of iron to catalyze protein changes with different fish (Tokur *et al.,* 2007).

Generally, disulfide bonds increased in all samples, regardless of iron concentration (Figure 3c). The increase in disulfide bond content was correlated ($R^2=0.92$) with the decrease in sulfhydryl content (Figure 3b). However, with the 7th cycle, a higher disulfide bond content was found with iron (5-25 ppm) compared to controls ($P<0.05$). Conversion of sulfhydryl groups into disulfides and other oxidized species can be due to radical-mediated oxidation of protein (Dean, 1997). Decreasing sulfhydryl group content of common carp (*Cyprinus carpio*) surimi as influenced by frozen storage and multiple freeze-thaw cycles has been reported (Kong *et al.,* 2013; Li *et al.,* 2013). Polymerization of high molecular weight protein via disulfide bonding in fish muscle induced by iron was also reported (Tokur *et al.,* 2007).

Changes in Ca^{2+}-ATPase Activity

Ca^{2+}-ATPase activity is shown in Figure 4. A decrease in Ca^{2+}-ATPase activity of all samples with increasing freeze-thaw cycles was observed. Decreasing Ca^{2+}-ATPase activity might be due to the conformational changes and protein polymerization. A decline in Ca^{2+}-ATPase activity of fish sample with increasing frozen storage time indicated myosin denaturation, especially in the head region (Benjakul *et al.,* 2000). Kobayashi and Park (2017) reported that Ca^{2+}-ATPase activity of NAM extracted from frozen tilapia (*Oreochromis niloticus*) decreased when frozen storage time increased. Decreasing Ca^{2+}-ATPase activity of NAM extracted from carp surimi induced by freeze-thawing has also been reported (Kong *et al.,* 2013). The results correlated with the increase in surface hydrophobicity ($R^2 = 0.95$) (Figure 3a) and disulfide bond content ($R^2 = 0.94$) (Figure 3c). However, there were no differences for Ca^{2+}-ATPase activity between iron and control samples during the first two cycles ($P\geq0.05$). Thereafter, slightly lower Ca^{2+}-ATPase activity with iron, compared to controls was found (Figure 4). These results suggested that iron affected Ca^{2+}-ATPase activity of Sawai pastes to some degree, specifically as freeze-thaw cycles increased.

Changes in Protein Solubility

Decreases in protein solubility were observed (Figure 5). The decreased solubility of Sawai pate samples during freeze-thawing correlated ($R^2 = 0.92$) with the decreased sulfhydryl group content (Figure 3b) and Ca^{2+}-ATPase activity (Figure 4) as well as the increased SoANS (Figure 3a) and disulfide bond content (Figure 3c). The decrease in protein solubility is used as an indicator of protein denaturation. This may be caused by the formation of hydrophobic and disulphide bonds (Campo-Deano, Tovar, & Borderias, 2010). After two freeze-thaw cycles, the samples containing iron showed lower protein solubility ($P<0.05$) than control which correlated ($R^2=0.99$) with lower Ca^{2+}-ATPase activity (Figure 4). Apart from the effect of freeze-thaw cycles, iron addition might directly affect protein solubility by binding to carboxyl groups. The decrease in protein solubility of common carp surimi during extended frozen storage has been reported (Li *et al.,* 2013). Changes in protein structure and functional properties such as protein polymerization and loss of solubility, caused by oxidation, have also been reported (Ooizumi & Xiong, 2004). Protein oxidation of some fish species (sardine, Atlantic bonito and bluefish) induced by iron addition has also been reported (Tokur *et al.,* 2007).

Changes Observed with SDS-PAGE

Protein patterns using SDS-PAGE are shown in Figure 6. The aggregation of Sawai protein induced by iron addition (5-25 ppm) can be seen as evidenced by the formation of high (higher than the myosin heavy chain, MHC) molecular weight polymers with concomitant decreases in myosin and actin intensities (Figure 6b). Protein aggregation was explained by the loss of sulfhydryls and the formation of disulfide bonds between or within polypeptides (Xia, Kong, Xiong, & Ren, 2010). The disappearance of polymers and the reappearance of myosin and actin in the presence of reducing agents suggested that the polymers were formed via disulfide linkages between proteins (Figure 6a). These results confirmed that iron accelerated protein oxidation in Sawai pastes, especially with 7 freeze-thaw cycles. Similar changes in electrophoretic patterns have also been reported (Thanonkaew *et al.,* 2006) in oxidized cuttlefish muscle. No fragmentation

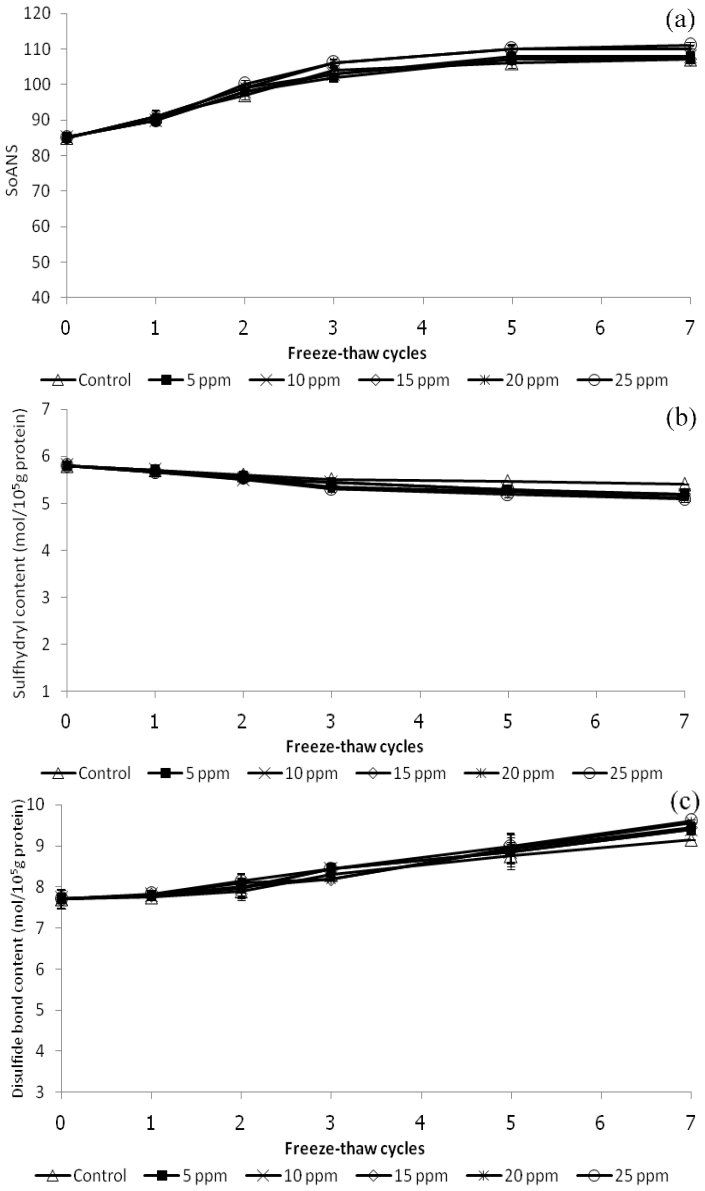

Figure 3. Changes in surface hydrophobicity (SoANS) (a) sulfhydryl content (b) and disulfide bond content (c) of natural actomyosin extracted from Sawai paste with different concentrations of iron during multiple freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

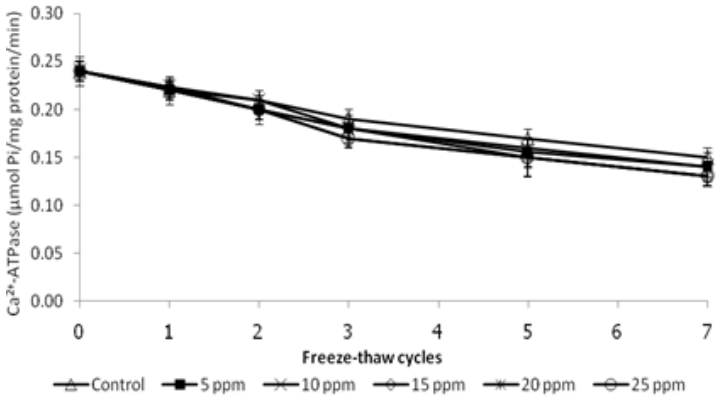

Figure 4. Changes in Ca^{2+}-ATPase activity of natural actomyosin extracted from Sawai paste with different concentrations of iron during multiple freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

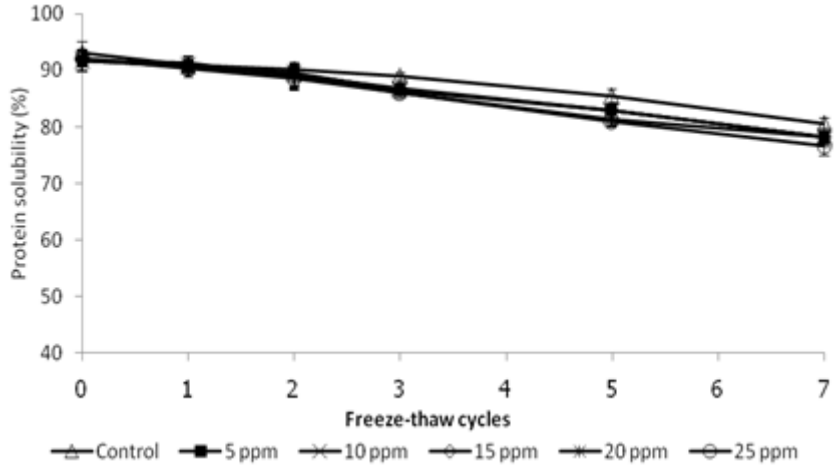

Figure 5. Changes in protein solubility of Sawai paste with different concentrations of iron during multiple freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

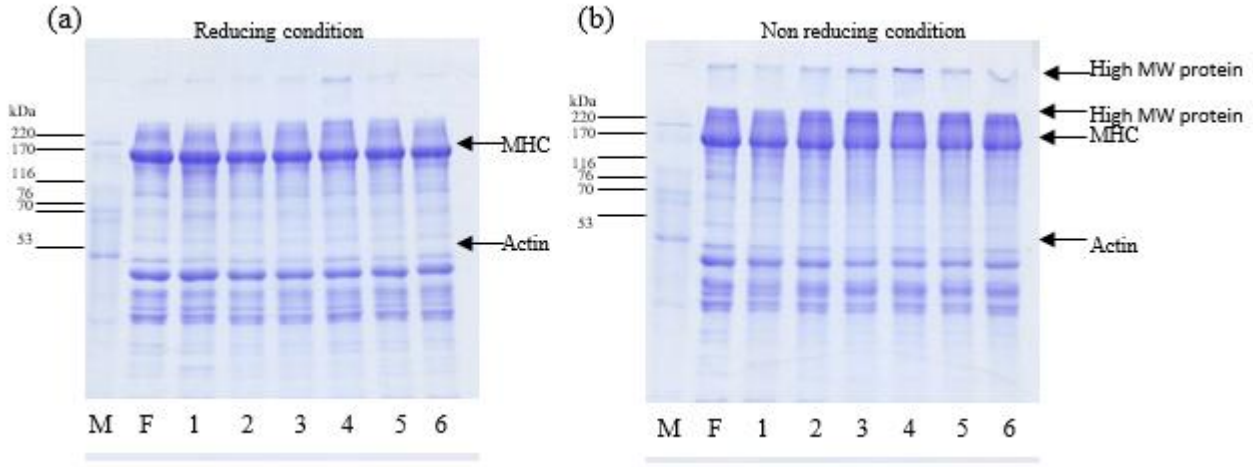

Figure 6. SDS-PAGE pattern of Sawai paste with different concentrations of iron (0-25 ppm) and subjected to multiple freeze-thaw cycles. F: fresh sample, 1: control sample (no iron addition), 2: sample with 5 ppm iron, 3: sample with 10 ppm iron, 4: sample with 15 ppm iron, 5: sample with 20 ppm iron, 6: sample with 25 ppm iron.

could be observed in any of the fish protein samples. Thanonkaew et al. (2006) also did not observe any fragmentation in oxidized cuttlefish protein.

Conclusion

The addition of iron to Sawai pastes increased lipid oxidation, yellowness (b* value) and the loss of protein functionality during freeze-thawing. Increased lipid oxidation and yellow color of Sawai pastes with iron addition was observed in a dose dependent manner. However, iron had only negligible effects on protein oxidation even with increasing freeze-thaw cycles. The formation of a yellow color (increasing b* values) was due to iron addition, suggesting that lipid oxidation is closely related to yellow discoloration. Therefore, to maintain fish quality, the prevention of the yellow discoloration induced by lipid oxidation should continue to be studied.

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