

CHAPTER 7

THE EFFECT OF ANTIOXIDANTS ON THE QUALITY CHANGES OF CUTTLEFISH (*SEPIA PHARAONIS*) MUSCLE DURING FROZEN STORAGE

7.1 Abstract

The changes in quality of cuttlefish (*Sepia pharaonis*) treated with 5% NaCl and 0.3% H₂O₂ and soaked without and with different antioxidants during frozen storage at -18 °C for 16 weeks were investigated. Thiobarbituric acid reactive substances (TBARS) in all cuttlefish samples increased when the storage time increased (p<0.05). Ascorbate (ASC) and erythorbate (ERT) showed the prooxidative effect while EDTA and tripolyphosphate (TPP) had no antioxidative effect in frozen cuttlefish. Soaking the cuttlefish in 5% NaCl and 0.3% H₂O₂ for 15 min could improve the color of cuttlefish by increasing the L*-value and decreasing a*-value. ASC, ERT, EDTA and TPP solutions had no impact on a*-value and L*-value of cuttlefish during frozen storage. However, the treated samples, which were soaked in ASC and ERT solutions had the increased b*-value during frozen storage. S₀ANS of cuttlefish increased when the frozen storage period increased up to 12 weeks. The increase in disulfide bond content was generally coincidental with the decrease in sulfhydryl content. ASC, ERT, EDTA and TPP had no pronounced effect on those changes. Protein solubility decreased slightly during prolonged storage. Soaking cuttlefish with 5% NaCl and 0.3% H₂O₂ together with 0.5% TPP could retard the decreases in solubility and to prevent thaw drip of frozen cuttlefish. However, ASC, ERT and EDTA showed no impact on the solubility and thaw drip of frozen cuttlefish.

7.2 Introduction

Frozen and thawed fish products are in general characterized by having lower quality than fresh ones due to the osmotic removal of water, denaturation of protein and

mechanical damage (Thyholt and Isaksson, 1997). Lipid oxidation occurring during frozen storage leads to the formation of free radicals and hydroperoxides, which cause oxidation of pigments, flavors and vitamins. Lipid oxidation products such as ketones, aldehydes, alcohols, hydrocarbons, acids and epoxides are formed during the oxidation of unsaturated fatty acids, producing off-flavor and reacting with protein to produce off-color (Khayat and Schwall, 1983). Lipid oxidation processes lead to discoloration, drip losses, and off-flavor development (Decker and Hultin, 1992) and production of potentially toxic compounds (Xiong, 2000). Furthermore, lipid oxidation in fish muscle during frozen storage showed the detrimental effect on protein structure and functionality (Saeed and Howell, 2002). Protein and lipid oxidation can account for the toughened texture, poor flavor and/or unappealing odor of poorly stored frozen seafood (Khayat and Schwall, 1983). Norwegian spring-spawning herring turned to be yellow color when it was frozen. The changes of color are correlated with an increase in TBARS and peroxide values (Hamre *et al.*, 2003). Recently, lipid oxidation was reported to promote formation of yellow pigment in cuttlefish mince with repeated freeze-thawing (Thanonkaew *et al.*, 2006a) and in squid microsome and liposome systems (Thanonkaew *et al.*, 2006b).

Prevention of oxidative deterioration of muscle food during processing or storage can be achieved by the incorporation of antioxidants (Decker, 1998). Ascorbate and erythorbate are strong reducing agents and have been successfully used as an antioxidant in many foods including meat products. Ascorbic acid efficiently scavenges hydrogen peroxide and free radicals. It may also restore vitamin E by reducing the tocopheroxyl radical to its native state (Igoe and Hui, 2001). However ascorbate can also reduce Fe(III) to Fe(II) and Cu(II) to Cu(I), thereby increasing the prooxidant activity of these metals (Buettner, 1993). Thus, it can serve as both a prooxidant and an antioxidant. Chelating agents can form a complex with the unwanted trace metals, thus blocking the reactive sites of the metal ions and rendering them inactive. Chelating agents including EDTA, phosphates and citric acid have been used to prevent deterioration of color and texture as well as to prevent lipid oxidation. Phosphates have been widely accepted as potential additives in meat and seafood to improve the functional properties of those products by increasing water retention especially in frozen-thawed meat and seafood products (Chang and Regenstein, 1997). Phosphates inactivate metal ions either by decreasing

metal solubility or by complexing and maintaining them in a soluble redox inactive (Ellinger, 1975).

Quality deterioration due to discoloration, poor texture, development of off-flavors and yellow discoloration in frozen cuttlefish causes the rejection of the product. Those changes may be associated with lipid oxidation of cuttlefish, whose lipids contained high content of phospholipids. Therefore, the use of antioxidants could retard the quality losses of frozen cuttlefish. This study aimed to investigate the stabilizing effect of some antioxidants on lipid oxidation, discoloration, and protein denaturation of cuttlefish (*Sepia pharaonis*) muscle during frozen storage.

7.3 Materials and Methods

Chemicals

1-anilinonaphthalene-8-sulphonic acid (ANS), 5-5'-dithio-bis (2-nitrobenzoic acid) (DTNB), β -mercaptoethanol (β -ME), cysteine, sodium bisulfite, L-ascorbic acid (sodium ascorbate) and sodium D-isoascorbate (sodium erythorbate) were obtained from Sigma (St. Louis, MO, USA). Sodium chloride, hydrogen peroxide, ethylene diamine tetraacetic acid (EDTA) and sodium tripolyphosphate (TPP) were purchased from Merck (Darmstadt, Germany). Acrylamide and bis-acrylamide were procured from Fluka (Buchs, Switzerland).

Cuttlefish collection and preparation

Cuttlefish (*Sepia pharaonis*), caught by cast net from Songkhla coast along the Gulf of Thailand and offloaded about 24 h after capture, were purchased from a dock in Songkhla province. Samples with the size of 8-10 cuttlefish/kg were placed in ice with a sample/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 1 h. The cuttlefish were washed, deskinning, eviscerated and the eyes were removed. The prepared cuttlefish were washed, drained and kept in ice during preparation.

Treatment of cuttlefish with various antioxidants

Prepared cuttlefish were cut into $4 \times 4 \text{ cm}^2$ and the samples (1 kg) were soaked in 5% NaCl containing 0.3% H_2O_2 (4°C) at a ratio of 1:1 (w/v) for 15 min with an occasional stirring. Thereafter, the treated samples were drained and soaked in different antioxidant solutions (4°C) at a ratio of 1:1 (w/v) for 15 min as follows: 1) 0.5% ascorbate (ASC), 2) 0.5% erythorbate (ERT), 3) 0.5% ethylene diamine tetraacetic acid (EDTA) and 4) 0.5% tripolyphosphate (TPP). After soaking, the samples were drained at 4°C for 5 min. Each treatment (150 g) was placed in polyethylene bags, frozen and stored at -18°C . Samples without any treatment were used as the control. Analyses of the frozen cuttlefish were carried out at 0, 2, 4, 8, 12 and 16 weeks.

Thawing of frozen cuttlefish

Frozen cuttlefish were thawed using running tap water ($25\text{-}27^\circ\text{C}$) until the core temperature reached $0\text{-}2^\circ\text{C}$. Thawed cuttlefish were measured for thaw drip and color. Thawed cuttlefish were cut, finely chopped and used for chemical and physicochemical analysis. The samples were kept in ice during analysis.

Preparation of natural actomyosin

Natural actomyosin (NAM) was prepared according to the methods of Benjakul *et al.* (1997). Cuttlefish paste was homogenized (IKA Labortechnik, Salangor, Malaysia) in 10 volumes of 0.6 M KCl (4°C) for totally 4 min on ice. Homogenization was performed in 20 sec bursts, followed by 20 sec rest periods to avoid over heating. The homogenate was centrifuged at 5000 xg for 30 min at 4°C using a RC-5B Plus Centrifuge (Sorvall, Norwalk, CT, USA) and the supernatant, to which 3 volumes of chilled water were added, was collected. The diluted supernatant was centrifuged at 5000 xg for 30 min at 4°C to precipitate the NAM. NAM was solubilized in 0.6 M KCl (1:1) and then recentrifuged at 5000 xg for 30 min at 4°C to precipitate

insoluble protein. The supernatant was collected and the protein content was adjusted to 4 mg/mL. The NAM solution was used for analysis of surface hydrophobicity, total sulfhydryl and disulfide bond contents.

Surface hydrophobicity

Surface hydrophobicity (S_0 ANS) was determined as described by Benjakul *et al.* (1997) using 1-anilinonaphthalene-8-sulphonic acid (ANS) as a probe. NAM solution (4 mg/ml) was diluted in 10 mM phosphate buffer, pH 6.0, containing 0.6 M NaCl to obtain protein concentrations of 0.1%, 0.2%, 0.3% and 0.5%, followed by incubation at room temperature for 10 min. The diluted protein solution (2 mL) was mixed with 20 μ L of 8 mM ANS in 0.1 M phosphate buffer, pH 7.0 and the fluorescence intensity of ANS-conjugates was immediately measured 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 S_0 ANS.

Total sulfhydryl content

The total sulfhydryl content was determined using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) according to the method of Ellman (1959) as modified by Benjakul *et al.* (1997). One mL of NAM solution (4 mg/mL) 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 a 0.6 M KCl solution as a blank. The sulfhydryl content was calculated using the extinction coefficient of 13500 $M^{-1} cm^{-1}$.

Disulfide bond content

The disulfide bond content was determined using the 2-nitro-5-thiosulfobenzoate (NTSB) assay according to the method of Thannhauser *et al.* (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 dark at room temperature for 25 min. The absorbance at 412 nm was measured. The disulfide bond content was calculated using the extinction coefficient of $13900 \text{ M}^{-1} \text{ cm}^{-1}$.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according the method of Laemmli (1970). The cuttlefish samples were solubilized in 5% SDS and dissolved in sample buffer with and without β -mercaptoethanol. The samples (20 μ g protein) were load into the polyacrylamide gel made of 4% stacking gel and 10% running gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protein II unit (Bio-Rad Laboratories, Inc, Richmond, CA, USA). After separation, the protein were stained with 0.02% Coomassie Brilliant Blue R-250 in 50% methanol and 7.5% acetic acid and detained with 50% methanol and 7.5% acetic acid, followed by 5% methanol and 7.5% acetic acid.

Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) were determined as described by Buege and Aust (1978). Finely chopped samples (2 g) were 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 xg for 20 min at room temperature. The absorbance of the supernatant was measured at 532 nm. The standard curve was prepared using malonaldehyde and TBARS were expressed as mg malonaldehyde/kg sample.

Protein solubility

Solubility was determined according to Benjakul and Bauer (2000). To 1 g of cuttlefish sample, 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 (Selangor, Malaysia). The homogenate was stirred at 4 °C for 4 h, followed by centrifuging at 8500 xg for 30 min at 4 °C using a Biofuge Primo centrifuge (Sorvall, Hanau, Germany). To 10 mL of supernatant, cold 50% (w/v) trichloroacetic acid was added to obtain a final concentration of 10%. The precipitate was washed with 10 volumes of 10% trichloroacetic acid and solubilized in 0.5 M NaOH. The cuttlefish sample was also directly solubilized by 0.5 M NaOH to obtain total protein. Protein content was determined using the Biuret method (Robinson and Hodgen, 1940).

Thaw drip

The weight of cuttlefish was recorded before freezing and frozen storage. After thawing process, the thawed samples were weighed and the amount of thaw drip was calculated according to the following formula (Santos and Regenstein, 1990):

$$\% \text{ Thaw drip} = [(A-B)/A] \times 100$$

where A = Initial weight of sample; B = Final weight of sample

Color

The color of the cuttlefish was measured using a colorimeter (Hunter Lab, Model Color Flex, Virginia, USA) and reported in the CIE color profile system as L*-value (lightness), a*-value (redness/greenness) and b*-value (yellowness/blueness).

Statistical analysis

Completely randomized design (CRD) was used in this study. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) (Steel and Torrie, 1980). Statistical analysis was performed

using the Statistical Package for Social Science (SPSS 10.0 for windows, SPSS Inc., Chicago, IL).

7.4 Results and Discussion

Changes in lipid oxidation

Changes in the TBARS values of cuttlefish during frozen storage at -18°C for 16 weeks are shown Figure 33. The formation of TBARS in cuttlefish increased when the frozen storage time increased, especially after 12 weeks of storage. Among all samples, those treated with 5% NaCl and 0.3% H_2O_2 , which is the condition commonly used in cuttlefish processing, tended to have the lowest value with the extended storage. Salt solution is used to make the texture firm, whereas H_2O_2 is applied for bleaching purpose. Ascorbate (ASC) and erythorbate (ERT) showed the prooxidative effect in frozen cuttlefish. TBARS values of cuttlefish soaked with ASC and ERT increased more rapidly than other samples, particularly after 4 weeks of frozen storage ($p < 0.05$). From the result, ASC and ERT might reduce ferric state or cupric state in cuttlefish to the high prooxidative ferrous state or cuprous state. The ferrous state or cuprous state of metal accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via Fenton type reaction (Dunford, 1987). ASC can act as a prooxidant at low (0.02-0.03%) concentrations and as an antioxidant at high (0.5%) concentrations (Decker and Xu, 1998). Mitsumoto *et al.* (1991) reported that the addition of 500 ppm of ascorbic acid to ground beef decreased lipid peroxidation. Nevertheless, Benedict *et al.* (1975) found that 50 ppm ascorbic acid caused the increased lipid peroxidation in ground beef. From the result, EDTA and TPP did not show the antioxidative effect in frozen cuttlefish throughout the storage. It was presumed that prooxidative metal ions in the intact muscle might not be scavenged completely. As a result, lipid oxidation still took place as evidenced by the formation of TBARS. Cho *et al.* (2001) compared the oxidative stabilities of lipids from the viscera, muscle, and eye of squid with those of other kinds of marine lipids and found that oxidative stabilities of these lipids were higher than that of tuna orbital lipid, bonito oil and trout egg lipid, respectively.

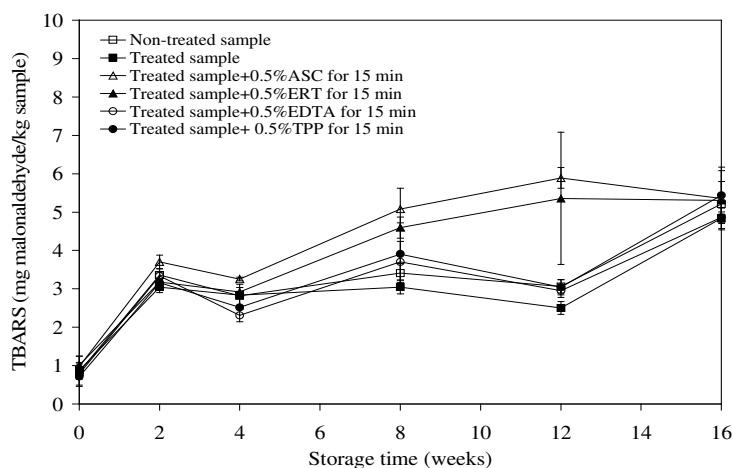


Figure 33. Thiobarbituric acid reactive substances (TBARS) in cuttlefish treated with 5% NaCl and 0.3% H₂O₂, followed by soaking in different antioxidant solutions during frozen storage at -18 °C for 16 weeks. Error bars indicate the standard deviations from triplicate determinations.

Changes in color

Color plays an important part in the appearance, presentation and acceptability of frozen cephalopod. Generally, color changes can occur during frozen storage due to lipid oxidation and pigment degradation process (Daias *et al.*, 1994). L*, a* and b* -values of frozen cuttlefish treated with 5% NaCl and 0.3% H₂O₂ and soaked with and without antioxidants soaking are shown in Figure 34. L*-value of all samples slightly decreased at 4 weeks of storage. Thereafter, L*-value was stable up to 16 weeks of frozen storage. Non-treated cuttlefish had lower L*-value but higher a*-value than other samples ($p < 0.05$). Therefore, soaking solution (5% NaCl and 0.3% H₂O₂ for 15 min) could improve the color of cuttlefish by increasing the L*-value and decreasing a*-value. H₂O₂ is well known in food as antimicrobial or bleaching agent.

H₂O₂ is widely used in cephalopod industry as the bleaching agent. The cephalopod needs to be bleached because the flesh of cephalopod could be stained by ink, viscera and color pigments during handling and processing. The decomposition of hydrogen peroxide gives the oxygen radicals (oxidizing agents), which can react with many substances (Perkins, 1996). Oxidizing agents were able to break the chemical bonds that make up the chromophore. This changes the molecule into a different substance that either does not contain a chromophore, or contains a chromophore that does not absorb visible light (Perkins, 1996).

ASC, ERT, EDTA and TPP had no impact on L*-value and a*-value of cuttlefish during frozen storage. However, ASC and ERT-treated samples increased in b*-value with increasing storage time, especially after 8 weeks of frozen storage ($p < 0.05$). Among all samples, ASC-treated sample had the highest b*-value ($p < 0.05$). A positive correlation between lipid oxidation (TBARS) and yellow color formation was found in the samples treated with ASC and ERT. An increase in lipid oxidation in cuttlefish mince during freeze-thaw cycling was coincidental with an increase in b*-value (Thanonkaew *et al.*, 2006a). In squid microsome and liposome system, a decrease in a*-value (red color) and an increase in b*-value (yellow color) were observed with the concomitant increase in TBARS formation (Thanonkaew *et al.*, 2005; Thanonkaew *et al.*, 2006b). Yu *et al.*, (2002) also found that the increase of lipid oxidation in cooked turkey muscle was correlated with the decrease in redness and the increase in yellowness. These results suggested that yellow pigment formation in cuttlefish could be due to nonenzymatic browning reactions between lipid oxidation products and the amine in phospholipid head groups or the amine in protein (Thanonkaew *et al.*, 2006b). Phospholipids are the major lipid in cuttlefish and contained a high content of PUFA (Thanonkaew *et al.*, 2006c). Thus, cuttlefish phospholipids most likely underwent oxidation rapidly during the extended frozen storage.

Changes in physicochemical properties of natural actomyosin

Changes in surface hydrophobicity (S_0 ANS) indicate conformational changes in protein structure. The changes in S_0 ANS of NAM extracted from treated cuttlefish with and without antioxidants are depicted in Figure 35. S_0 ANS of cuttlefish NAM increased when the frozen storage period increased up to 12 weeks. However, at 16 weeks of frozen storage, S_0 ANS

of cuttlefish NAM were slightly decreased. ASC, ERT, EDTA and TPP did not affect S_0 ANS of NAM from frozen cuttlefish ($p > 0.05$). An increase in surface hydrophobicity possibly resulted from structural alterations of proteins induced by frozen storage. During extended frozen storage, the proteins underwent conformational changes, in which the hydrophobic and hydrogen bonds buried inside the protein molecules become exposed and broken from their native structure. As a consequence, conformational changes in coiled or helical section of the peptide chain could occur and reform in a manner different from those in the native structure (Morawetz, 1972). The enhanced hydrophobicity was coincidental with the increased oxidation of lipids, suggesting that protein structural changes might result from the modifications of amino acid residue side chains by lipid free radicals or fatty acid decomposition products. Hydrophobic interaction might take place between the exposed hydrophobic residues, leading to the aggregation protein. Thus, frozen storage directly affected the conformational changes in protein molecules.

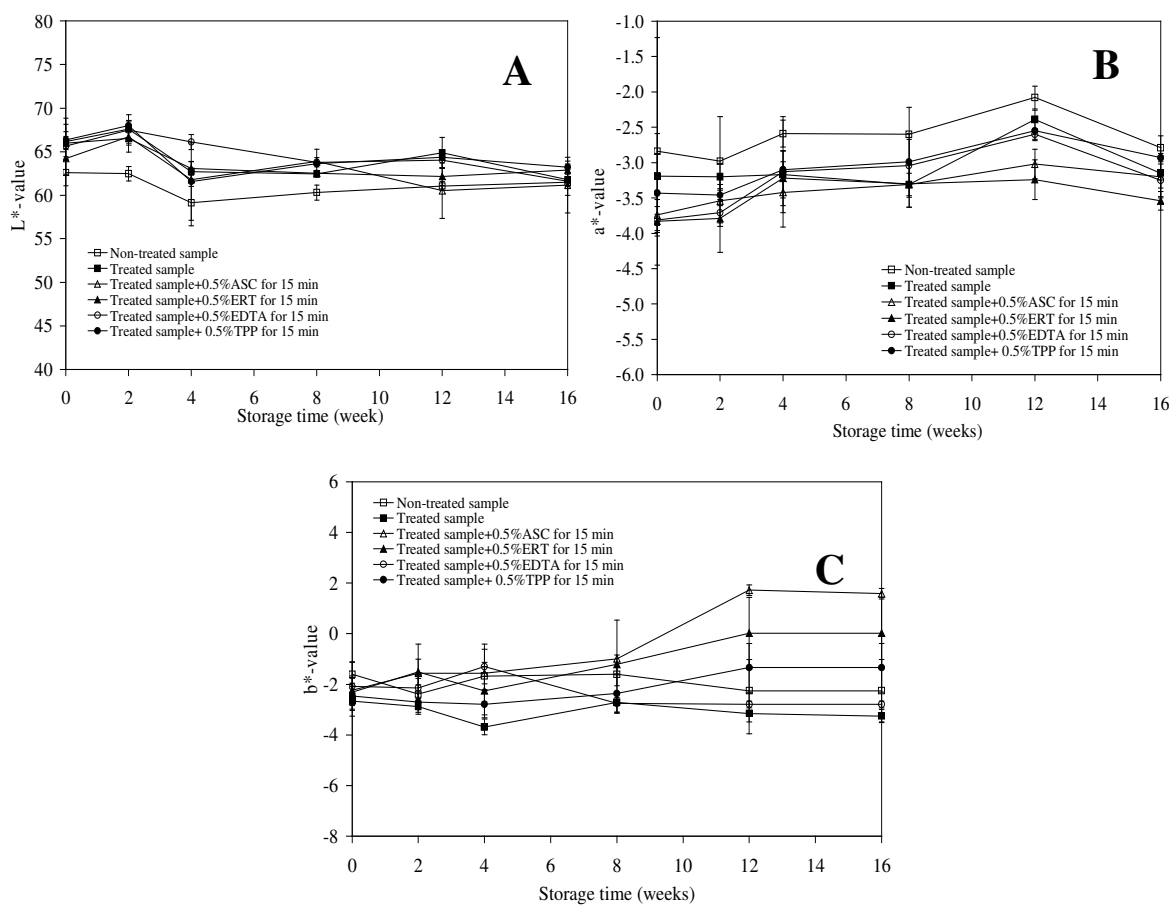


Figure 34. L* (A), a* (B) and b* (C)-values of cuttlefish treated with 5% NaCl and 0.3% H₂O₂, followed by soaking in different antioxidant solutions during frozen storage at -18 °C for 16 weeks. Error bars indicate the standard deviations from triplicate determinations.

Sulfhydryl and disulfide bond contents of NAM from cuttlefish with different treatments are shown in Figure 36. Sulfhydryl content of NAM from cuttlefish decreased with the concomitant increase in disulfide bond content during frozen storage up to 16 weeks ($p < 0.05$). During frozen storage, the conformational change of protein molecules might be associated with the exposure of reactive sulfhydryl groups, which were prone to oxidation or disulfide interchange. Under normal frozen storage conditions, muscle proteins also undergo instability (Benjakul and Bauer, 2000). Myosin is susceptible to oxidizing agents during processing and storage. The disappearance of both heavy and light chains of myosin is much more pronounced than is actin and tropomyosin (Xiong, 2000). In general, the denaturation and aggregation of protein started from the formation of disulfide bonds, followed by a rearrangement of hydrophobic and hydrogen-bonded regions on an intra- and inter-molecular basis (Buttkus, 1970). From the results, ASC, ERT, EDTA and TPP exhibited no marked effect on sulfhydryl and disulfide bond contents of cuttlefish, when compared with non-treated sample. Since those compounds did not show the antioxidant effect on lipid oxidation, the radicals could be produced and initiated the protein oxidation at the same rate with the control (non-treated). Conversion of sulfhydryl groups into disulfides and other oxidized species is one of the radical-mediated oxidation of protein (Dean *et al.*, 1997).

Under both non-reducing (without β -mercaptoethanol) and reducing conditions, similar SDS-PAGE patterns of cuttlefish and cuttlefish treated with 5% NaCl and 0.3% H₂O₂ and soaked with ASC, ERT, EDTA solutions were obtained (Figure 37). Three major protein bands were observed in cuttlefish muscle, corresponding to myosin heavy chain (MHC), actin and paramyosin. The result was in accordance with that reported by Thanonkaew *et al.* (2006a). However, the band intensity of all proteins was more retained under non-reducing condition. The

result suggested that disulfide bond involved in cross linking of proteins in both fresh and frozen/treated samples. Disulfide bonds could be formed in cuttlefish muscle during extended frozen storage (Figure 36B). Recently, Thanonkaew *et al.* (2006a) reported that both Cu(I) and Cu(II) effectively catalyzed protein denaturation and caused dramatic changes in cuttlefish muscle proteins by the formation of disulfide bond.

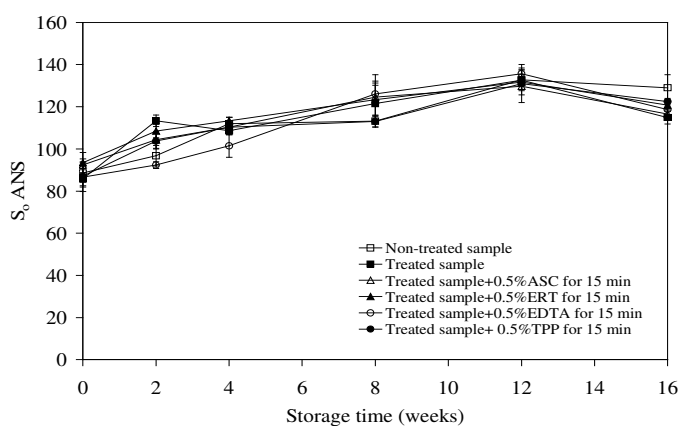


Figure 35. Surface hydrophobicity (S_0ANS) of natural actomyosin extracted from cuttlefish treated with 5% NaCl and 0.3% H_2O_2 , followed by soaking in different antioxidant solutions during frozen storage at $-18\text{ }^{\circ}\text{C}$ for 16 weeks. Error bars indicate the standard deviations from triplicate determinations.

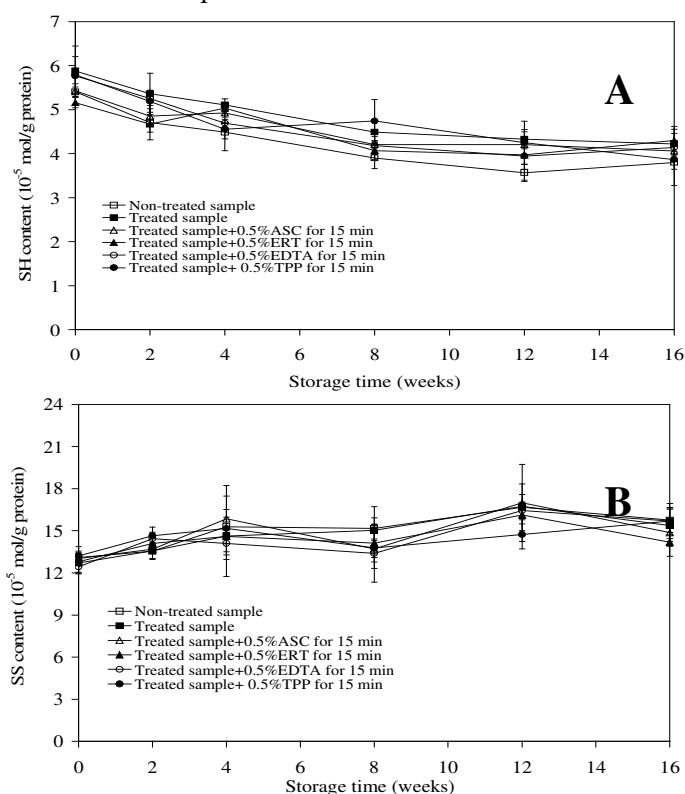


Figure 36. Sulphydryl content (A) and disulfide bond content (B) of natural actomyosin extracted from cuttlefish treated with 5% NaCl and 0.3% H₂O₂, followed by soaking in different antioxidant solutions during frozen storage at -18 °C for 16 weeks. Error bars indicate the standard deviations from triplicate determinations.

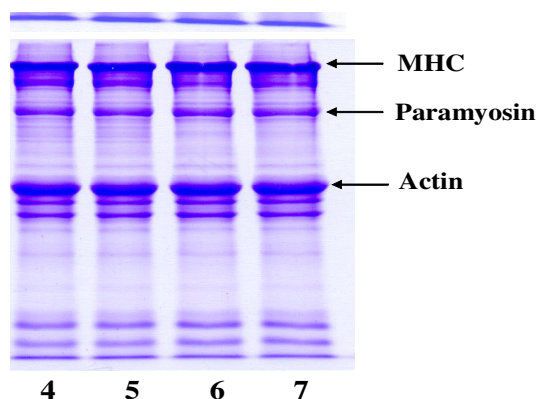


Figure 37. SDS-PAGE pattern of cuttlefish treated with 5% NaCl and 0.3% H₂O₂, followed by soaking in different antioxidant solutions during frozen storage at -18 °C for 16 weeks, lane 1: non frozen cuttlefish, 2: Control (non-treated), lane 3: soaking solution, lane 4: ASC, 5: ERT, 6: EDTA and 7: TPP.

Changes in solubility

Protein solubility of cuttlefish and those with different treatments in 0.6 M KCl during frozen storage is shown in Figure 38. Protein solubility decreased slightly during the first 8 weeks of storage. However, solubility of non-treated cuttlefish decreased rapidly after 8 weeks of frozen storage ($p < 0.05$). After 12 weeks of frozen storage, solubility of non-treated cuttlefish was decreased to 71.83%, while solubility of cuttlefish treated with 5% NaCl and 0.3% H₂O₂ and soaked without and with ASC, ERT, EDTA and TPP solution were decreased to 77.67%, 76.75%, 85.58%, 85.32% and 90%, respectively. Salt could increase the water-holding capacity of meat products through extraction of salt-soluble meat protein (Rhee, 1999). During frozen storage, cuttlefish treated with NaCl could imbibe more water and the migration of water to form ice

crystals was retarded. As a result, less aggregation of protein occurred. The decrease in salt-soluble protein concentrations of cuttlefish during frozen storage was coincidental with the increase in S_0 ANS (Figure 35), loss of sulfhydryl content and disulfide bond formation (Figure 36). A gradual decrease in protein extractability during frozen storage of squid (*Loligo duvauceli*) and voladur (*Illex coindentii*) was also reported (Joseph *et al.*, 1997; Ruiz-Capillas *et al.*, 2002). The decrease in solubility of cuttlefish might be caused by protein denaturation and protein aggregation induced by freezing and frozen storage. Thermodynamically, a decrease in protein solubility is the result of a shift from a tendency of proteins to interact with water towards a situation where proteins interact with each other (Vojdani, 1996). Freezing reduces the solubility of proteins, especially the myofibrillar proteins, which are generally extracted with 0.6 M NaCl. From the result, the solubility of cuttlefish treated with 5% NaCl and 0.3% H_2O_2 , followed by soaking with 0.5% TPP was maintained throughout the storage of 16 weeks. Therefore, phosphate, which could increase water holding capacity, of cuttlefish muscle, effectively retarded the decrease in solubility. In presence of phosphate, protein-water interaction could be maintained and protein-protein interaction was more likely prevented. ASC, ERT and EDTA did not exhibit the positive effect in maintaining the solubility of frozen cuttlefish throughout the storage.

Changes in thaw drip

The lost moisture, known as thaw drip, normally occurs in fish and carries with the large portion of the soluble protein, mineral and others nutrients (Gillies, 1975). Thaw drip in frozen cuttlefish and cuttlefish with different treatments is shown in Figure 39. Thaw drip of all samples increased as the storage time increased up to 8 weeks ($p < 0.05$). Thereafter, no changes were observed until 16 weeks of frozen storage ($p > 0.05$). At 16 weeks of storage, the highest thaw drip was found with non-treated cuttlefish (13.3%), whereas cuttlefish treated with 5% NaCl and 0.3% H_2O_2 , followed by soaking with 0.5% TPP tended to have the lowest thaw drip (8.75%). Thaw drip of treated samples and soaked with ASC, ERT and EDTA solutions were 11.47%, 10.34% and 9.48%, respectively. Therefore, TPP significantly decreased the thawing drip of frozen cuttlefish. The enhanced hydration power in phosphate-meat is attributed to the

remarkable swelling of myofibril architecture and extraction of actomyosin (Xiong, 1999). It is a common practice in the fish industry to drip scaled and filleted or skinned and filleted fish in sodium chloride brine to reduce the moisture loss during thawing, to enhance the palatability of the fish and to reduce the thaw drip (Gillies, 1975). The use of phosphate alone or together with salt significantly reduces thawing drip, tendency to turn yellow and susceptibility to the development of rancidity (Gillies, 1975). Continuous denaturation of proteins in cuttlefish muscle during frozen storage led to the lower water holding capacity of proteins. Ice crystal formed resulted in the tissue damage and the leakage of various organelles. As a result, water could be released from muscle more easily, particularly when the frozen storage time increased (Benjakul *et al.*, 2003). From the result, soaking the treated sample with 0.5% TPP resulted in the reduced thaw drip of frozen cuttlefish. However, other compounds including ASC, ERT and EDTA showed no marked influence on improving the water holding capacity of sample treated with 5% NaCl and 0.3% H₂O₂.

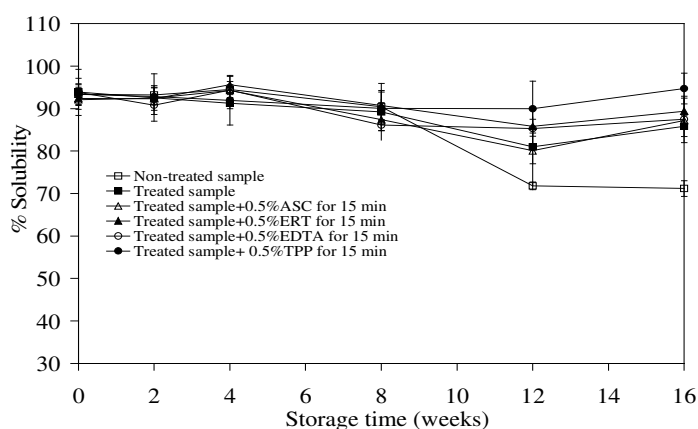


Figure 38. Protein solubility of cuttlefish treated with 5% NaCl and 0.3% H₂O₂, followed by soaking in different antioxidant solutions during frozen storage at -18 °C for 16 weeks. Error bars indicate the standard deviations from triplicate determinations.

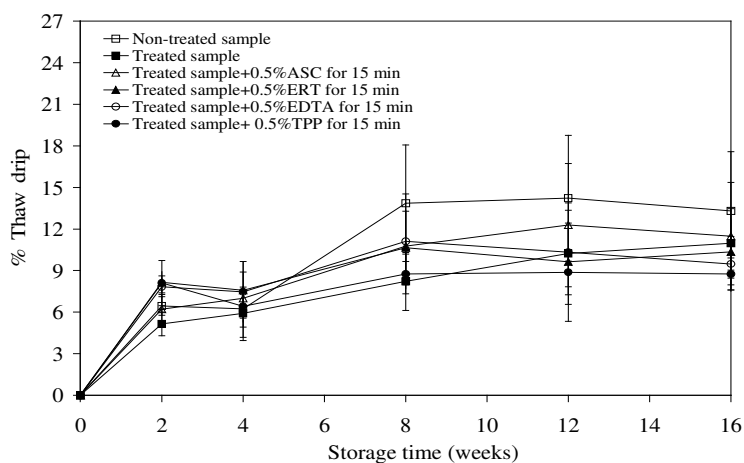


Figure 39. Thaw drip of cuttlefish treated with 5% NaCl and 0.3% H₂O₂, followed by soaking in different antioxidant solutions during frozen storage at -18 °C for 16 weeks. Error bars indicate the standard deviations from triplicate determinations.

7.5 Conclusion

All antioxidants (ASC, ERT, EDTA and TPP) did not prevent lipid oxidation in frozen cuttlefish. Conversely, ASC and ERT exhibited the prooxidative activity and increased the yellow color formation in frozen cuttlefish. The treatment of cuttlefish with soaking solution (5% NaCl and 0.3% H₂O₂) together with 0.5% TPP was an important process to improve the color and reduce the decrease in solubility and the thaw drip of frozen cuttlefish.