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Influence of Washing and Cold Storage on Lipid and Protein Oxidation in Catfish (Clarias lazera) Surimi

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ABSTRACT
Lipid and protein oxidation in catfish (Clarias lazera) surimi during processing and storage were assessed. Catfish surimi were washed in deionized water: M0 (no washing step), M1 (one washing step), and M2 (two washing steps). Lipid, protein, water, and iron contents were determined. M0, M1, and M2 were stored for 0, 1, 4, 7, or 10 days at 4 ± 1°C; at each time point, samples were removed for analyses. Lipid oxidation was assessed by measuring malondialdehyde content. Protein oxidation was assessed by measuring protein solubility and protein sulfhydryl and carbonyl group contents. Based on the results, lipid content, \( L^* \) and \( a^* \) (color parameters), and fatty acid content in M1 and M2 were significantly reduced. Lipid oxidation development was faster in M1, and the ranking was as follows: M1 > M2 > M0, with M0 being significantly less oxidized than M1. Increasing the number of washes increased protein oxidation, and the ranking was as follows: M2 > M1 > M0. Altogether, lipid and protein oxidation and physicochemical changes occurred simultaneously to different degrees in surimi during various processing and storage conditions.

KEYWORDS
Catfish; surimi; storage; protein oxidation; lipid oxidation

Introduction
In the past years, there has been an increasing demand for processed surimi products, which, compared to red meat products, contain a higher content of omega-3 polyunsaturated fatty acids (n-3 PUFAs) such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). Catfish (Clarias lazera) is a fatty fish with a fat content of 11–14% (Zhen et al., 2008). In 1981, catfish was imported from Egypt into Guangdong Province, China. Catfish, which is in high demand, is characterized by rapid growth rates, high yields, less demanding breeding conditions, low susceptibility to diseases, and high protein contents, especially sarcoplasmic and dark muscle proteins (Zhen et al., 2008).

Surimi products are becoming very popular in China (Zhen et al., 2008). Surimi, a fish protein concentrate obtained by successive washings of fish mince, is obtained during the production of seafood products such as crab sticks and fish meatballs (Okada, 1992). The production of fish mince from fatty fish species has the potential to provide healthy fish products with appreciable levels of omega-3 fatty acids. Due to their high fat content and the high amount of sarcoplasmic proteins and dark muscle, surimi-related foods such as fish ball, sausage, fish tofu, etc., have been investigated in...
order to obtain surimi with no odor, light color, and good gel forming ability with sources from fatty fish species (Duan and Shan, 2007; Hultin and Kelleher, 2000; Ren et al., 2012). Surimi products have many advantages—e.g., high nutritional value; ease of preparation; and a wide variety of sources, types, and sizes, among others.

However, during processing and storage, the quality of fatty fish products may decline as a result of reactions that affect both protein and lipid molecules and that decrease the nutritional and sensory properties of the products. Fatty fish products contain high levels of unsaturated fatty acids, which are very susceptible to oxidation and thus to the formation of free radicals and lipid hydroperoxides. Free radicals and lipid hydroperoxides, which are primary lipid oxidation products, are converted into secondary lipid oxidation compounds such as alcohols, aldehydes, and ketones. Lipid oxidation contributes to the formation of unpleasant odors, rancid taste, and discoloration (Frankel, 2005; Deng et al., 2011). Moreover, lipid oxidation can modify proteins through cross-linking reactions, thereby affecting amino acids and decreasing protein functionality (e.g., solubility and hydrophobicity) as a result of protein denaturation (Pokorny et al., 1976). Muscle proteins can be modified not only by reactive oxygen species (ROS) including free radicals, but also by nonradical oxygen species such as H$_2$O$_2$ and lipid hydroperoxides. Oxidation reactions lead to the formation of free radicals, amino acid derivatives, and protein denaturation and polymerization, which negatively impact the functional properties of the product such as its texture and gel forming properties (Xiong, 2000; Soyer et al., 2010).

The objective of this study was to assess the oxidation reactions in lipid and protein fractions during the processing and storage of catfish surimi. This study would allow a more thorough understanding of the oxidation reactions and their effects on surimi quality. By repeatedly washing surimi, it is possible to produce surimi containing different amounts of lipid and protein content and also to assess their susceptibility to oxidation.

**Materials and methods**

**Reagents and standards**

All chemicals at analytical grade, were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and from Shnopham Chemical Reagent Co. Ltd. (Beijing, P.R. China). The chemicals used in gas chromatography (GC) were obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, P.R. China). Deionized water (Millipore system) was used throughout the experiments. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and standards were obtained from Sigma Chemical Co.

**Raw material, processing, and storage conditions**

Approximately 1,000–1,500 g of catfish was purchased from the wholesale market in Tianjin, China and transported to the Department of Food Science of the Tianjin Agricultural University (approximately, a 30 min drive). All processing steps were performed in a 4°C temperature-controlled room. Catfish were submerged in ice-cold water (5–7°C for 10 min), hand-skinned, gutted, and ground using a grinder plate with 3 mm holes (Mincer, Zhucheng Jinshan Machinery Co. Ltd., Shandong, P. R. China). The mince was washed twice in cold distilled water (4°C, pH 6.85) at a 1:3 (w/v) ratio. Samples were slowly stirred for 30 s every 2 min for 4 min and then allowed to settle for 6 min. Each washing step lasted 10 min. Following each washing step, the fish surimi was dried for approximately 5 min between two layers of gauze (Eymard et al., 2009). The three types of surimi products obtained were: (a) catfish mince (M0) obtained after grinding, (b) catfish surimi obtained after the first drying step (M1), and (c) catfish surimi obtained after the second drying step (M2). Portions (220 g sample for every storage time) of M0, M1, and M2 were packaged in plastic bags (16 cm × 19 cm), which were sealed under vacuum (Vacuum sealer, Zhucheng Meichuan Machinery Co. Ltd., Shandong,
P.R. China) and stored at 4 ± 1°C for 0, 1, 4, 7, or 10 days. At each storage time point, a portion of the catfish surimi product was removed and stored at −80°C for 10 days until further analysis.

**Proximate analysis**

**Water content**

Approximately 2 g of sample was weighed after drying (115°C for 12 h) in order for water content to be quantified (AOAC, 1995). Analyses were performed in triplicate, and results were expressed as g of water per 100 g of wet sample.

**Total lipid content**

Total lipids were extracted in triplicate from 10 g of sample using a mixture of methanol and chloroform (1:2, v/v), according to Bligh and Dyer (1959). The lipid content was determined gravimetrically of partition in chloroform phase via solvent evaporation, and the results were expressed as g of lipid/100 g of wet sample.

**Total protein content**

Total protein was determined by Kjeldahl method according to the AOAC (2001) method. The N factor is 6.25; the protein content was determined, and the results were expressed as g of protein/100 g of wet sample.

**Iron content**

The iron content in catfish surimi was determined using a Shimadzu AA-6300 atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan). Catfish muscle (2 g) was mixed with 5 mL of concentrated HNO$_3$ and 2 mL of H$_2$O$_2$. The samples were acid-digested for 25 min in a microwave oven. After the acid-digestion step, the samples were analyzed according to the Nordic Committee on Food Analysis (1991) protocol.

**Fatty acid composition**

The fatty acid composition in the M0, M1, and M2 lipid extracts was determined by GC of fatty acid methyl esters (FAMEs), according to the AOAC (2001) Official Method No. 996.06.

Catfish surimi (30–40 g) was mixed with 90 mL of petroleum ether and allowed to stand for more than 9–12 h. Subsequently, the mixture was filtered with two layers of gauze, and the filtrate was placed in 60°C water bath for lipid extraction (Zang et al., 2006). Approximately 0.1 mL of the lipid extract and 1 mL of standard (100 mg of n-heptadecane-methyl dissolved in 50 mL of n-hexane) were placed in a 10 mL volumetric flask, followed by the addition of 2 mL of benzene-petroleum ether extract (1:1). The solution was mixed and allowed to stand for 30 min; 1 mL of 0.4 mol/L KOH (with methanol as solvent) was then added. The resulting FAMEs were either injected into the GC column or transferred to an auto-sampler vial for GC analysis.

A DB-WAX quartz capillary column (30 m × 0.25 mm) was used. The stationary phase consisted of a free fatty acid phase (FFAP). The detector and injection port temperatures were both set at 280°C. The column temperature was set at 180°C for the first 20 min and then increased to 200°C at a rate of 0.5°C/min. The gas mobile carrier consisted of N$_2$ (25 mL/min) and H$_2$ (40 mL/min); the air velocity was 450 mL/min with a split ratio of 30:1.

**Preparation of myofibrillar proteins**

Myofibrillar proteins were extracted according to the method by Xiong (2000) with a slight modification. Surimi (5 g) was homogenized at 13,000 rpm for 30 s at 4°C in four times the volume of protein extract (containing 0.1 mol/L NaCl, 2 mmol/L MgCl$_2$, 1 mmol/L EGTA, and 10 mmol/L
Na₂HPO₄). After the homogenization step, the mixture was centrifuged at 2,000 × g for 15 min at 4°C, and the resulting supernatant was discarded. The process was repeated three times. The myofibrillar proteins were kept in ice for 48 h.

**Myofibrillar protein solubility**

The solubility of the myofibrillar proteins was assessed by the method reported by Benjakul and Baher (2000) with a slight modification. Samples (0.5 g) were homogenized for 1 min in 10 mL of 0.6 M KCl with 50 mM Tris-HCl buffer at pH 7.4 using a Polytron FLUKO FA25 High Shear Homogenizer (FLUKO, Shanghai, P.R. China). The homogenate was centrifuged at 14,000 × g for 15 min at 5°C. The protein content in the supernatant was determined by the Biuret method (Bradford, 1976). Percent solubility (PS) was expressed as the ratio of protein content in the supernatant of myofibrillar protein samples to that in the fresh sample.

**Determination of sulfhydryl group content**

Approximately 0.5 mL of a protein sample solution (2 mg/mL) was placed in a 10 mL centrifuge tube. Then, 2.5 mL of 8 M urea in Tris-Gly (10.4 g Tris, 6.9 g glycine, and 1.2 g EDTA in 1 L of water, pH 8.0) and 0.02 mL of Ellman’s reagent—5,5'-dithiobis-(2-nitrobenzoic acid)—in Tris-Gly (4 mg/mL) were added. The solution was kept in the dark for 25 min. Absorbance was immediately measured at 412 nm (Shimadzu UV-1800). The sulfhydryl (SH) group content was calculated using a molar extinction coefficient of 13,600 M⁻¹ cm⁻¹ (Ellman, 1959). Results were expressed in nmoles of SH/mg of protein. SH was calculated using the following formula:

\[
SH(n\text{ mole/mg~protein}) = \frac{10^6 \times A_{412}}{\varepsilon \times C},
\]

where \(A_{412}\) is the absorbance at 412 nm; \(C\) is the sample concentration in mg protein/mL; \(\varepsilon\) is the molar absorption coefficient of 13,600 M⁻¹ cm⁻¹; and \(10^6\) is for conversions from the millimolar basis to the nM/mL basis and from mg protein to g protein.

**Determination of carbonyl content**

Carbonyl groups were detected with 2,4-dinitrophenylhydrazine (DNPH), which results in the formation of protein hydrazones, using the method reported by Oliver et al. (1987) with slight modifications. A myofibrillar protein solution (25 mg/mL) was precipitated with 10% trichloroacetic acid (TCA; w/v; final concentration). After centrifugation (2,000 × g for 10 min), the resulting pellet was treated with 0.2% DNPH (w/v) in 2 M HCl and mixed for 1 h at room temperature. The solutions were then precipitated with 10% TCA (final concentration) and centrifuged (11,000 × g for 3 min). The pellets were washed twice with 1 mL of ethanol:ethyl acetate (1:1 v/v), and the solution was precipitated with 10% TCA (final concentration) and centrifuged. Proteins were then dissolved in 2 mL of 6 M guanidine containing 20 mM sodium phosphate buffer at pH 6.5. Absorbance of the DNPH-treated samples was measured at 365 nm (Shimadzu UV-1800); 2 M HCl was used as the control. Protein concentration in 2 M HCl was calculated at 280 nm using bovine serum albumin (BSA) in guanidine as standards. The carbonyl content was expressed as nmol of DNPH/mg of protein using an absorption coefficient of 22,000 M⁻¹ cm⁻¹ for the protein hydrazones.

**Determination of TBARS**

The determination of thiobarbituric acid reactive substances (TBARS) was performed according to the method reported by Vyncké (1975) with slight modifications. In a plug-graduated tube, 5 g of sample and 15 mL of 7.5% TCA, 0.1% BHA, and 0.1% EDTA were mixed for 30 s in a high-speed
homogenizer (10,000 RPM). The mixture was then filtered through a Φ7 cm qualitative filter paper (the pore size is 30–50 μm); 2.5 mL of the resulting filtrate was mixed with 2.5 mL of 0.02 mol/L of 2-thiobarbituric acid and placed in a boiling water bath for 40 min. Following the 40-min incubation, the solution was placed in ice and mixed with 5 mL of chloroform. This solution was centrifuged at 8,000 × g for 10 min; the absorbance of the supernatant was measured at 532 nm (Shimadzu UV-1800). TBARS was calculated using the following formula:

\[ TBARS (\text{mg/kg}) = \frac{A \times V \times M}{\varepsilon \times 1 \times m}, \]

where \( A \) is the absorbance; \( V \) is the sample volume (mL); \( M \) is the molecular weight of malondialdehyde (i.e., 72.063); \( \varepsilon \) is the molar absorption coefficient of 156,000 M\(^{-1}\) cm\(^{-1}\); \( 1 \) represents the optical path length (cm); and \( m \) is the sample weight (g).

**Gel electrophoresis (SDS-PAGE)**

Using the method described by Laemmli (1970), SDS-PAGE can be used to detect denatured and polymerized proteins. Myofibrillar protein solution—2 mg/mL, the protein concentration was measured by the Bradford (1976) method—was dissolved in an appropriate amount of SDS-PAGE sample buffer (4% SDS, 20% glycerol, 0.125 M Tris-HCl, with or without 10% β-mercaptoethanol, pH 6.8) to obtain a 1 mg/mL protein concentration. Where β-mercaptoethanol was not used, 0.5 mM of N-ethylmaleimide (a thiol blocking agent) was added to the SDS-PAGE protein samples before boiling water heating to prevent disulfide artifacts (Xia et al., 2009). Aliquots of 20 μL of the samples were loaded to the wells in the stacking gel. The SDS-PAGE consisted of a 4% stacking gel and a 12% separating gel. A molecular weight (MW) standard, consisting of a cocktail of proteins of known MWs (14–200 kDa; TaKaRa Biotechnology, Dalian Co. Ltd., Dalian, P.R. China) was also loaded to the gel and run together with the samples. Constant current at 20 mA for stacking gel and 40 mA for separating gel were used for electrophoresis.

**Color determination**

The color of the M0, M1, and M2 surimi samples was measured using a colorimeter (Hunter Lab, Cincinnati, OH, USA). The instrument was calibrated using a white standard plate (\( L^* = 95.13, a^* = -0.09, \) and \( b^* = 3.39 \)). The values, expressed as \( \Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \), \( L^* \) (lightness), \( a^* \) (redness), and \( b^* \) (yellowness), were determined from three different sections on the upper surface of the surimi samples.

**Statistical analyses**

The experiment was replicated twice with at least triplicate analyses each time. Data were analyzed using the Statistix 8.1 package Linear Models (Analytical Software, Tallahassee, FL, USA), and the drawing software consisted of Sigma Plot 10.0 (Softonic International, S.A., Barcelona, Spain). Tukey’s honestly significant difference (HSD) test was used. Statistical significance was set at \( p < 0.05 \).

**Results and discussion**

**Characterization of the different surimi samples at day 0**

Water content varied between 68.77% in M0 and 74.50% in M2 (Table 1). In the process of washing, the rinse water combined with surimi gradually; thus, the water content increased with the increase of washing times. The results are consistent with the previous study by Eymard et al. (2009). A large
lipid fraction was removed during the washing steps. The lipid contents were 13.47, 10.29, and 8.89 g/100 g of wet sample in M0, M1, and M2, respectively (Table 1). Our current result is consistent with the research on horse mackerel (Trachurus trachurus) by Eymard et al. (2009), which suggested that washing can remove partial lipid fraction. The neutral lipids, which represent the storage lipids, were more easily removed during processing; this finding was in accordance with the results of previous studies (Eymard et al., 2005).

The percentage of saturated fatty acids in the surimi relative to the total fatty acids was 59–71%. The highest and lowest percentage of monounsaturated fatty acids were obtained in M1 (39.50%) and M2 (29.43%), respectively. Similarly, the highest percentage of PUFAs (mainly C18:2n6c) was obtained in M1 (0.79%); the PUFA percentage in M2 was under the detection limit (Table 1). Fat floating in the rinse water surfaced in the process of rinsing; with the increase of rinse times, fat and the rinse water were removed, and the total fatty acid content decreased. The total fatty acid content was 3,170.5 mg/100 g for M0, but it decreased to 620.33 and 371.46 mg/100 g for M1 and M2, respectively (Table 1). In catfish, the most abundant saturated fatty acid was C16:0, followed by C18:0. The most abundant monounsaturated fatty acid was C18:1n9c, whereas C18:2n6c was the most abundant PUFA. There was no EPA (C22:5n-3) or DHA (C22:6n-3) detected in the catfish. The type and content of fatty acids decreased with the number of washes, which is in accordance with results from a previous study (Eymard et al., 2009), showing washing can remove partial fatty acids.

The content of iron, a strong pro-oxidant, was determined in M0, M1, and M2. During processing, iron content decreased from 5.003 µg/g in M0 to 2.736 µg/g in M2 (Table 1). Myoglobin, a soluble pigment protein responsible for meat color, contains iron. Both myoglobin and iron content decreased with the number of washes, which is in accordance with results from previous studies.
The high proportion of red muscle tissue and, consequently, of heme proteins (e.g., hemoglobin and myoglobin) explained the high iron content in M0. The degree of iron loss significantly occurred in the first wash as it reduced from 5.003 to 3.21 µg/g wet sample (35.8%) compared to 14.8% in the second wash due to the removal of hemoglobin and myoglobin. This finding is in accordance with the results obtained by Chaijan et al. (2004) on washed sardine and mackerel samples.

Certain physicochemical properties of proteins changed during the washes of the surimi. The proportion of soluble proteins decreased from 39.95% in M0 to 35.04% in M2. TBARS increased from 0.07 mg/kg in M0 to 0.211 and 0.113 mg/kg in M1 and M2, respectively. This result suggests that washing affects lipid oxidation during storage (Table 1). This finding, however, was not in agreement with the results reported by Eymard et al. (2009). The discrepancy in the results might be attributed to different packaging methods (e.g., wrapped tray versus vacuum packaging). In our study, M1 had the highest TBARS value. The level of α-tocopherol (vitamin E), a natural antioxidant, decreased during processing (Eymard et al., 2009). In M1, the lipid content was very high (10.29 g/100 g) and the endogenous vitamin E (an antioxidant) was removed with the first wash. In M0, both the lipid and vitamin E content was high. In M2, vitamin E was removed by the washes, but the lipid content was relatively low compared to M1. Therefore, compared to M0 and M2, M1 was more susceptible to lipid oxidation. Based on the lipid oxidation susceptibility, M1 > M2 > M0 with M0 being less susceptible to lipid oxidation than M1 and M2. The order of protein oxidation was different from that of the lipid oxidation. In the case of protein oxidation, the order was M2 > M1 > M0 with M0 being less susceptible to protein oxidation than M2 and M1.

In the process of washing, surimi was mixed with distilled water and filtered. The surimi kept in contact with oxygen in the air, and with the increase of washing times, protein oxidation degree increased. The contents of carbonyl and SH groups, which are both protein oxidation markers, increased and decreased, respectively, during catfish processing. Carbonyl content increased from 0.524 nmol/mg protein in M0 to 1.855 nmol/mg protein in M2; whereas SH group content decreased from 51.175 nmol/mg protein in M0 to 43.986 nmol/mg protein in M2 (Table 1). Therefore, in these surimi samples, there was not a simple correlation between lipid oxidation and protein oxidation. A faster production of secondary lipid oxidation products (measured as TBARS) compared to protein carbonyl compounds has previously been observed in pork and beef patties stored in similar high oxygen conditions (Ma and Xiong, 2011; Lund et al., 2007).

L*, a*, b*, and ΔE* values of M0, M1, and M2 are shown in Table 1. With increasing number of washes, a* decreased from 11.42 to 3.44 (p < 0.05), and L* increased from 34.23 to 42.24 (p < 0.05). However, b* was not affected by the number of washes; its value remained the same. These results are probably due to the removal of sample pigments after the first wash. The significant difference in a* values among the samples suggests that washing significantly reduced myoglobin and hemoglobin content. However, the washes did not significantly affect the b* value of surimi.

In summary, the results suggest that both lipid and protein oxidation took place during catfish processing. The level of lipid oxidation was the highest in M1 followed by M2 and M0, with M0 being significantly less oxidized than M1 and M2.

**Lipid oxidation during storage at 4 ± 1°C**

The TBARS of the surimi samples during cold storage are shown in Figure 1. The oxidation of samples (measured as TBARS) was significantly influenced by the duration of storage (p < 0.05). During refrigeration-temperature storage, the TBARS in M1 increased significantly; however, in M2 there was a slight increase (p < 0.05) in TBARS. During storage, the TBARS in M1 were consistently higher than those in M0 and M2; the TBARS in M2 were higher than those in M0 (Figure 1). This result was attributed to the presence of vitamin E (i.e., tocopherol) in M0 resulting in low TBARS values. Because vitamin E is lost during processing, the TBARS values in M1 increased with storage. In M2, large amounts of lipids were removed during processing, therefore contributing to low
TBARS values. Researchers have reported that the last washing step results in the removal of lipid oxidation products, thereby resulting in a product with low TBARS values (Eymard et al., 2005).

**Protein oxidation during storage at 4 ± 1°C**

Similar to lipid oxidation, protein oxidation is different among the three groups of catfish surimi. The oxidative deterioration of proteins in M0, M1, and M2 during storage is shown in Figure 2.

The formation of protein carbonyl compounds is one of the most important effects of protein oxidation (Sabeena Farvin et al., 2012). At T0, the lowest protein carbonyl content was obtained in M0 (0.524 nmol/mg of protein); however, protein oxidation took place very rapidly during storage at 4 ± 1°C, and the carbonyl group content reached its maximum value after 4 days of storage (Figure 2). In the washed surimi (i.e., M1 and M2), there was a significant increase in carbonyl group of M1 and M2 from Day 0 (1.655, 1.855 nmol/mg of protein) to Day 1 (1.988, 2.234 nmol/mg of protein) and from Day 1 to Day 4 (2.302, 2.333 nmol/mg of protein). The carbonyl content of M1 and M2 remained unchanged basically after Day 4 (Figure 2). Protein oxidation took place rapidly during the storage of M0, due to high heme protein (high iron content; Table 1), whereas it remained fairly stable during the storage of M1 and M2. Some amino acids—e.g., lysine, histidine, proline, and arginine—yield carbonyl compounds. Therefore, the content of carbonyl groups is a meaningful indicator of oxidative stress in muscle proteins (Dalle-Donne et al., 2003). Some studies have reported that during freezing storage of rainbow trout there is no increase in protein carbonyl groups (Baron et al., 2007), whereas other studies have reported an increase in protein carbonyl groups (Passi et al., 2005). The content of heme protein, which is known to induce protein oxidation, was high in M0; thus it is possible that the increase in protein carbonyl groups during storage could have been induced by heme proteins.

Conversion of SH groups into disulfides and other oxidized species is one of the earliest events during the free radical oxidation of proteins (Dean et al., 1997). SH group content decreased during storage for all samples (M0, M1, and M2), which is indicative of disulfide bond formation (Figure 2). At the beginning of storage, the SH group content in M0 was the highest among all three samples. A considerable reduction in the SH group content of all samples was observed during Day 0 to Day 4 of storage; subsequently, SH group content decreased. There was a slight difference in the SH group content between M1 and M2; the SH group content of M0 was consistently higher than that of M1.
and M2 (Figure 2). The loss of the SH groups may be attributed to the formation of disulfide bonds either within or between polypeptides. The reduction in the SH group content may also be due to degradation reactions. During the washing steps, surimi was stirred, thereby increasing the chance of surimi protein oxidation and thus reducing SH group content.

Myofibrillar proteins are salt-soluble proteins, which require a high ionic strength (> 0.4) for proper solubilization. However, salting out effect of myofibrillar proteins precipitation will happen when the ionic strength is high to a certain level. Protein solubility is indicative of the protein function and protein characteristic. As shown in Figure 3, the myofibrillar protein solubility in M0, M1, and M2 (stored at 4 ± 1°C) had a downward trend especially after 7 days of storage. At T0 and during the entire storage period, the protein solubility of the three samples was the greatest in M0 followed by M1 and M2 (Figure 3). Free radical attack is a major cause of decreased protein solubility (Decker et al., 1993). As shown, the reduction in protein solubility has been used as an indicator of muscle protein oxidative deterioration (Decker et al., 1993; Xiong and Decker, 1995). Because the ordered tertiary structure is lost, cross-linkages are formed among proteins, and thus protein solubility decreases. Here, we show that the decrease in protein solubility is also associated with an increase in the number of washes.

One of the main consequences of protein oxidation is the formation of protein aggregates and, in some cases, formation of protein fragments (Park et al., 2006). Figure 4 shows the SDS-PAGE result of myofibrillar proteins in M0, M1, and M2 stored at 4 ± 1°C. Irrelevant to the presence of β-mercaptoethanol (a disulfide bond breaking agent), significant changes in any of the protein bands were observed during surimi processing and storage at 4°C. The bands with molecular weight greater than 100 kDa, at 200 and 45 kDa for 10-day storage were darker and wider than those of other days, but these differences were not much evident. Concomitantly, some high MW polymers (protein aggregates, MW greater than 200 kDa), which stacked on the top of the separating gel, were observed. The results indicated that oxidation caused cross-linking of protein through disulfide bonds; however, the extent of such cross-linking was processing and storage time dependent. Ragnarsson and Regenstein (1989) showed that cross-linking of formaldehyde and dimethyamine via disulfide and nondisulfide covalent bonds during frozen storage contributed to the formation of high molecular weight polymers and aggregates. Protein cross-linking may affect
drop loss and tenderness, as the mechanism of water-holding in the form of binding and entrapping relates to the physical structures especially the structure of myofibrillar protein (Huff-Lonergan and Lonergan, 2005). Interestingly, fragmentation of myosin as reported previously (Liu and Xiong, 2000) was not observed under the present oxidative condition. This may be due to less degree protein oxidation occurring in our present study. The reason for this may be related to vacuum packaging, which might have prevented any reactions between the samples and oxygen and might have inhibited the growth of microorganisms. In the current study, the determination of protein sulfydryl and carbonyl clearly showed that myofibrillar proteins oxidized more with increasing washing times and storage time, and this oxidation was further demonstrated by the identification of cross-linked myofibrillar protein by SDS-PAGE.

**Conclusion**

After two washes (M2), the lipid content, $L^*$ and $a^*$ value, and fatty acid content was significantly reduced in catfish surimi. Increasing the number of washes increased protein oxidation. In terms of
lipid oxidation, surimi with one wash had the highest degree of lipid oxidation followed by surimi with two washes. In addition, M0, M1, and M2 protein oxidation increased, and M1 and M2 lipid oxidation increased during storage.

During storage, the rate of lipid oxidation was higher in M1 than in the other samples. The lipid oxidation rate ranking was M1 > M2 > M0. Lipid and protein oxidation occurred simultaneously in all three surimi samples during storage, although it is hard currently to determine at which level these two reactions took place.

In summary, this study indicated that during catfish surimi processing, the processing of washes affects surimi’s physiochemical properties and degree of lipid and protein oxidation. More washes correspond to higher lipid and protein oxidation. In addition, longer storage time is associated with increased level of lipid and protein oxidation. Future research is needed to understand the mechanism for how these occur. Catfish can be used in the surimi industry, although oxidation of lipid and protein occurs during the processing and storage. Thus, measures should be taken to prevent these events for catfish surimi production.

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**References**


