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Food Chemistry 98 (2006) 431–439
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of washing with oxidising agents on the gel-forming ability and physicochemical properties of surimi produced from bigeye snapper (*Priacanthus tayenus*)

Suttirug Phatcharat [a], Soottawat Benjakul [a,*], Wonnop Visessanguan [b]

[a] *Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[b] *National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Paholayothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand*

Received 28 March 2005; received in revised form 8 June 2005; accepted 8 June 2005

Abstract

The effects of washing with hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl) solutions on the gel-forming ability and physicochemical properties of surimi produced from bigeye snapper (*Priacanthus tayenus*), stored in ice for up to 14 days, were investigated. Generally, pH and the trichloroacetic acid (TCA)-soluble peptide content of washed mince varied, depending on the type of oxidizing agent and storage time of the fish. With increasing time of storage, the pHs of water- and H_2O_2-washed mince were lower than that of NaOCl-washed mince ($P < 0.05$). However, no differences in the TCA-soluble peptide contents of the resulting mince washed with any media were observed ($P > 0.05$). Washing with 20 ppm NaOCl resulted in the highest increase in both the breaking force and the deformation of mince from fish stored in ice for all the times studied ($P < 0.05$). Natural actomyosin (NAM) extracted from NaOCl-washed mince had higher surface hydrophobicity and disulfide bond (SS) content than that of water-washed mince ($P < 0.05$). With no effect on Ca^{2+}-, Mg^{2+}-, or Mg^{2+}–Ca^{2+}-ATPase activities, NaOCl washing resulted in an increase in Mg^{2+}–EGTA-ATPase activity of NAM ($P < 0.05$). The results suggested that washing mince with the appropriate type and concentration of oxidizing agent can improve the gelling ability of surimi, particularly from low quality fish.

Keywords: Surimi; Oxidising agents; Iced storage; Gel-forming ability; Mince

1. Introduction

Freshness of fish is generally considered as the most important factor determining the gel-forming ability of surimi. Time and temperature of storage of the fish between capture and processing can affect the final surimi quality (Park & Morrissey, 2000). Lower gel quality is generally associated with extended storage times in ice. However, the rate of loss of gel forming ability appears to vary among species (Benjakul, Visessanguan, Riebroy, Ishizaki, & Tanaka, 2002; Benjakul, Visessanguan, & Tueksuban, 2003a). The gel strength of kamaboko made from lizardfish kept in ice for 3 days was 50% of that made from fresh fish (Kurokawa, 1979). Northern squawfish surimi could be made from fish stored for up to 9 days (Lin & Morrissey, 1995). Generally, prolonged holding times and elevated temperatures can cause severe proteolysis of myofibrillar proteins, which directly results in an inferior gel quality (Suzuki, 1981). Degradation of myosin heavy chain also occurred during iced storage of Pacific whiting (Benjakul, Seymour, Morrissey, & An, 1997). During handling, leakage of digestive enzymes into the muscle contributes to the subsequent hydrolysis of muscle proteins. Therefore, pretreatment of fish, including beheading and eviscerating prior to handling or storage,

* Corresponding author. Tel.: +66 7428 6334; fax: +66 7421 2889.
E-mail address: soottawat.b@psu.ac.th (S. Benjakul).

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doi:10.1016/j.foodchem.2005.06.016

can be another way to retard the deterioration caused by proteolysis (Benjakul et al., 2002).

Thailand is one of the largest surimi producers in Southeast-Asia. Twelve surimi factories are located in Thailand with a total production of about 60,000 metric tons per year (Morrissey & Tan, 2000). Due to the insufficient amount of fresh fish, the surimi industry has to use low quality fish, which causes inferior gel quality. The improvement of gel properties has been achieved by various means, such as the addition of protein additives (Park & Morrissey, 2000), appropriate setting (Benjakul, Chantarasuwan, & Visessanguan, 2003b), and the use of microbial transglutaminase (Visessanguan, Benjakul, & Tanaka, 2003). Also, the washing process was shown to enhance the gel strength of surimi (Park & Morrissey, 2000). Since oxidising agents can induce the oxidation of proteins, particularly via disulfide formation (Liu & Xiong, 2000), protein fragments of degraded muscle proteins could be cross-linked and formed larger protein aggregates with improved gelling ability. This might be a feasible method to recover the gel-forming ability of low quality fish associated with the degradation.

Sodium hypochlorite (NaOCl) has a wide range of domestic, industrial, scientific and biomedical applications related to its biocide properties (US EPA, 1994). A food additive regulation permitting the use of sodium hypochlorites in washing or assisting in lye peeling of fruits and vegetables has been established (21 CFR 173.315) by the Food and Drug Administration (FDA). Hydrogen peroxide has been also used in food processing at the concentration of 0.04–1.25% (21 CFR 184.1366). Hydrogen peroxide degrades rapidly to oxygen and water. Therefore, residues in or on treated post-harvest food commodities are negligible. EPA has concluded that for food use, at an application rate of 1%, hydrogen peroxide has no apparent acute toxicity or subchronic toxicity. Additionally, hydrogen peroxide is listed by the Food and Drug Administration as Generally Recognized as Safe (GRAS). The maximum concentration of hydrogen peroxide used as an antimicrobial agent on meat carcasses, parts, trim and organs is 75 ppm (21 CFR 173.370). The objective of this study was to investigate the effect of washing with oxidising agents, including sodium hypochlorite and hydrogen peroxide, on the gel properties of surimi and physicochemical properties of natural actomyosin (NAM) extracted from washed mince produced from bigeye snapper (*Priacanthus tayenus*).

2. Materials and methods

2.1. Chemicals

Ammonium molybdate, 5-5′-dithio-bis(2-nitrobenzoic acid) (DTNB), adenosine 5-triphosphate (ATP), sodium dodecyl sulfate (SDS), calcium chloride and β-mercaptoethanol (βME) were purchased from Sigma (St. Louis, MO, USA). Sodium hypochlorite (NaOCl) was obtained from APS Ajax Finechem (NSW, Australia). Hydrogen peroxide (H_2O_2) and trichloroacetic acid were purchased from Riedel-deHaen (Seelze, Germany). All chemicals for electrophoresis were obtained from Bio-Rad (Richmond, CA, USA).

2.2. Fish sample

Bigeye snapper (*P. tayenus*), caught off the Songkhla-Pattani Coast along the Gulf of Thailand, and off-loaded 24 h after capture, were kept in ice using the fish/ice ratio of 1:2 (w/w). Fish were transported to the Department of Food Technology, Prince of Songkla University within 3 h. Upon arrival, fish were washed with tap water and kept in a styrene foam box containing crushed ice with a fish/ice ratio of 1:2 (w/w). The fish were placed and distributed uniformly between the layers of ice. The box was kept at room temperature (28–30 °C). To maintain the ice content, melted ice was removed and replaced with an equal amount of ice. Fish were randomly taken at days 0, 7, and 14 for chemical analyses and surimi preparation.

2.3. Surimi and surimi gel preparation

Fish kept in ice for different times were washed with tap water. The flesh was removed manually and minced to uniformity. The mince was then washed with different cold (5 °C) washing media (water, H_2O_2, NaOCl) at different concentrations (10, 20 and 40 ppm) using a mince/washing media ratio of 1:3 (w/v). The mixture was stirred gently for 5 min and the washed mince was filtered through two layers of cheesecloth. The washing process was repeated twice. Finally, the washed mince was centrifuged at 700*g* for 10 min with a model CE 21 K basket centrifuge (Grandiumpiant, Belluno, Italy). The washed mince was mixed with 4% (w/w) sorbitol and 4% (w/w) sucrose and kept at −18 °C until used.

To prepare the surimi gel, the frozen surimi was thawed using running tap water until the core temperature reached 0 °C. To the surimi, 2.5% (w/w) NaCl was added and the moisture content was adjusted to 80%. The mixture was chopped for 3.5 min at 4 °C to obtain a homogeneous sol. The sol was then stuffed into polyvinylidine casing with a diameter of 3.6 cm, and both ends of the casing were sealed tightly. Surimi gels were prepared by incubating the sol at 40 °C for 30 min, followed by heating at 90 °C for 20 min. The gels were cooled in iced water and stored overnight at 4 °C prior to analyses.

2.4. Determination of pH

The pH of washed mince was determined according to the method described by Benjakul et al. (2002). The

samples were homogenised using an IKA Labortechnik homogenizer (Selangor, Malaysia) with 5 volumes of distilled water (w/v), and the pH was measured using a pH meter (Cyberscan500, Singapore).

2.5. Determination of TCA-soluble peptides

TCA-soluble peptides were determined according to the method of Benjakul et al. (2002). Washed mince (3 g) was homogenised with 27 ml of 5% (w/v) TCA. The homogenate was kept in ice for 30 min and centrifuged at 5000*g* for 20 min, using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). Soluble peptides in the supernatant were measured according to the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) and expressed as μmol tyrosine g^{-1} muscle.

2.6. Determination of ATPase activities

ATPase activities were determined according to the method of Benjakul et al. (1997). Natural actomyosin (NAM), prepared as described by Benjakul et al. (1997), was diluted to 2.5–4 mg/ml with 0.6 M KCl, pH 7.0. One ml of the diluted solution was added to 0.6 ml of 0.5 M Tris–maleate, pH 7.0. To the mixture, one of the following solutions was then added for each ATPase activity assay to a total volume of 9.5 ml: 10 mM $CaCl_2$ for Ca^{2+}-ATPase, 2 mM $MgCl_2$ for Mg^{2+}-ATPase, 0.1 mM $CaCl_2$, 2 mM $MgCl_2$ for Mg^{2+}–Ca^{2+}-ATPase, 2 mM $MgCl_2$ and 0.5 mM EGTA for Mg^{2+}–EGTA-ATPase. To each assay solution, 0.5 ml of 20 mM ATP was added to initiate the reaction. The reaction was conducted for exactly 10 min at 25 °C and terminated by adding 5 ml of chilled 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 by the method of Fiske and Subbarow (1925). 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.

2.7. Determination of surface hydrophobicity

Surface hydrophobicity was determined as described by Benjakul et al. (1997), using 1-anililonaphthalene-8-sulphonic acid (ANS) as a probe. NAM dissolved in 10 mM phosphate buffer, pH 6.0, containing 0.6 M NaCl was diluted to 0.1%, 0.2%, 0.3%, and 0.5% (w/v) protein, using the same buffer. To the diluted protein solution (2 ml), 20 μl of 8 mM ANS in 0.1 M phosphate buffer, pH 7.0, were added. The fluorescence intensity of ANS-conjugates was measured using a RF-1501 spectrofluorometer (Shimadzu, Kyto, 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 NAM concentration was referred to as SoANS.

2.8. Determination of total sulfhydryl group and disulfide bond contents

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). To 1.0 ml of NAM solution (4.0 mg/ml), 9 ml of 0.2 M Tris–HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM EDTA, were added. To 4 ml of the mixture, 0.4 ml of 0.1% DTNB was added and incubated at 40 °C for 25 min. Absorbance at 412 nm was then measured. A blank was conducted by replacing the sample with 0.6 M KCl. Sulfhydryl content was calculated using the extinction coefficient of 13,500 $M^{-1}\,cm^{-1}$.

The disulfide bond content was determined using 2-nitro-5-thiosulfobenzoate (NTSB) assay, according to the method of Thannhauser, Konishi, and Scheraga (1987). To 0.5 ml of NAM sample (4.0 mg/ml), 3.0 ml of freshly prepared NTSB assay solution were added. The mixture was incubated in the dark at room temperature (25–30 °C) for 25 min. Absorbance at 412 nm was measured and the disulfide bond content was calculated using the extinction coefficient of 13,900 $M^{-1}\,cm^{-1}$.

2.9. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

The protein patterns of washed mince were analysed by SDS–PAGE, according to the method of Leammli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85 °C were added to the sample (3 g). The mixture was then homogenised for 2 min using an IKA homogenizer. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The samples were centrifuged at 3500*g* for 20 min to remove undissolved debris. Protein concentration was determined by the Lowry method (Lowry et al., 1951), using bovine serum albumin as standard. The SDS–PAGE gel was made of 10% running gel and 4% stacking gel. After separation, the proteins were fixed and stained with Coomassie Blue R-250.

2.10. Texture analysis

The texture of surimi gels was determined according to the method described by Benjakul et al. (2002). Gels were equilibrated and evaluated at room temperature (28–30 °C). Five cylinder-shaped samples with a length of 2.5 cm were prepared and subjected to determination. Breaking force and deformation were measured using a texture analyzer TA-XT2 (Stable Micro System, Surrey, UK) equipped with a cylindrical plunger (diameter 5 mm, depression speed 66 mm min^{-1}).

2.11. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test (Steel & Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 11.0 for windows: SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Effect on pH

The pH of washed mince varied, depending on the type of oxidising agent and storage time (Fig. 1). At day 0 of storage, mince washed with all media tested had pH values similar to that of *post-mortem* mince (pH 6.6–6.7). Benjakul et al. (2002) reported that the pH of ice-stored *P. tayenus* mince remained relatively constant for up to 6 days and gradually increased to pH 7.2, at day 9 of storage. In this study, the results showed that washing resulted in a marked decrease in pH, especially for the samples stored at day 14 ($P < 0.05$). With the extended time of storage, the pH of mince washed with NaOCl was higher than that of mince washed with H_2O_2. However, the pH of mince washed with water was lower than that of mince washed with both oxidising agents. The result suggested that those oxidising agents might induce the cross-linking of protein or those degradation bases, leading to the reduction of removal efficiency of those basic components. Nevertheless, water washing could generally leach out the small constituents, especially the decomposed products, including volatile bases, resulting in the decreased pH of the muscle. Generally, no marked differences in pH were observed between mince washed with the same washing media at different concentrations used.

3.2. Effect on TCA-soluble peptides

The type of washing medium had no effect on the TCA-soluble peptide content of the resulting washed mince ($P > 0.05$) (Fig. 2). However, washed mince obtained from extended iced storage generally had a higher content of TCA-soluble peptides. During iced storage, the TCA-soluble peptide content in *P. tayenus* muscle increased gradually up to 15 days (Benjakul et al., 2002). This result suggested that washing could not remove small peptides produced from the degradation process. Morrisey, Hartley, and An (1995) showed that proteolysis was detrimental to surimi quality by substantially lowering the gel strength. Proteolytic degradation of myofibrillar proteins, especially myosin, resulted in the reduction in molecular weight and the loss of structural domains, which are essential for molecular interaction and binding (Visessanguan & An, 2000). Even though cross-links and deposition of small fragments may occur, the resulting gel structures are much weaker than those of intact myosin, leading to lower gel strength.

3.3. Effect on properties and protein patterns of surimi gel

The breaking force of surimi gel prepared from mince washed with water or H_2O_2 decreased slightly after storage for 7–14 days (Fig. 3). The breaking forces of gels of surimi prepared by water washing were 624.6, 593.8 and 584.1 g at day 0, 7 and 14, respectively. Benjakul et al. (2002) reported that, when the storage time increased, the breaking force of surimi produced from both whole and headed/eviscerated bigeye snapper decreased up to 15 days of storage. This was coincidental with the increase in protein denaturation and degradation with increasing storage time (Benjakul et al., 2002).

Surimi, prepared by washing with H_2O_2 or NaOCl solutions at different concentrations (10, 20 and 40 ppm), showed a different breaking force ($P < 0.05$). At day 0, breaking force of surimi with 10 ppm H_2O_2

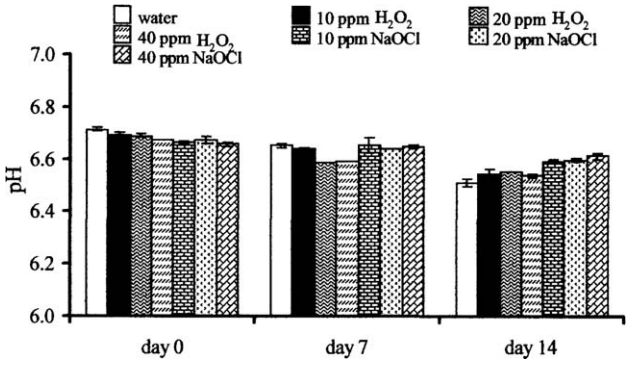

Fig. 1. Changes in pH of bigeye snapper mince stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from three determinations.

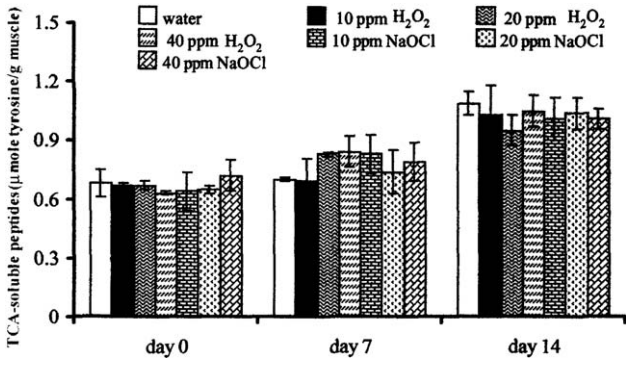

Fig. 2. Changes in TCA-soluble peptides in bigeye snapper mince stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from three determinations.

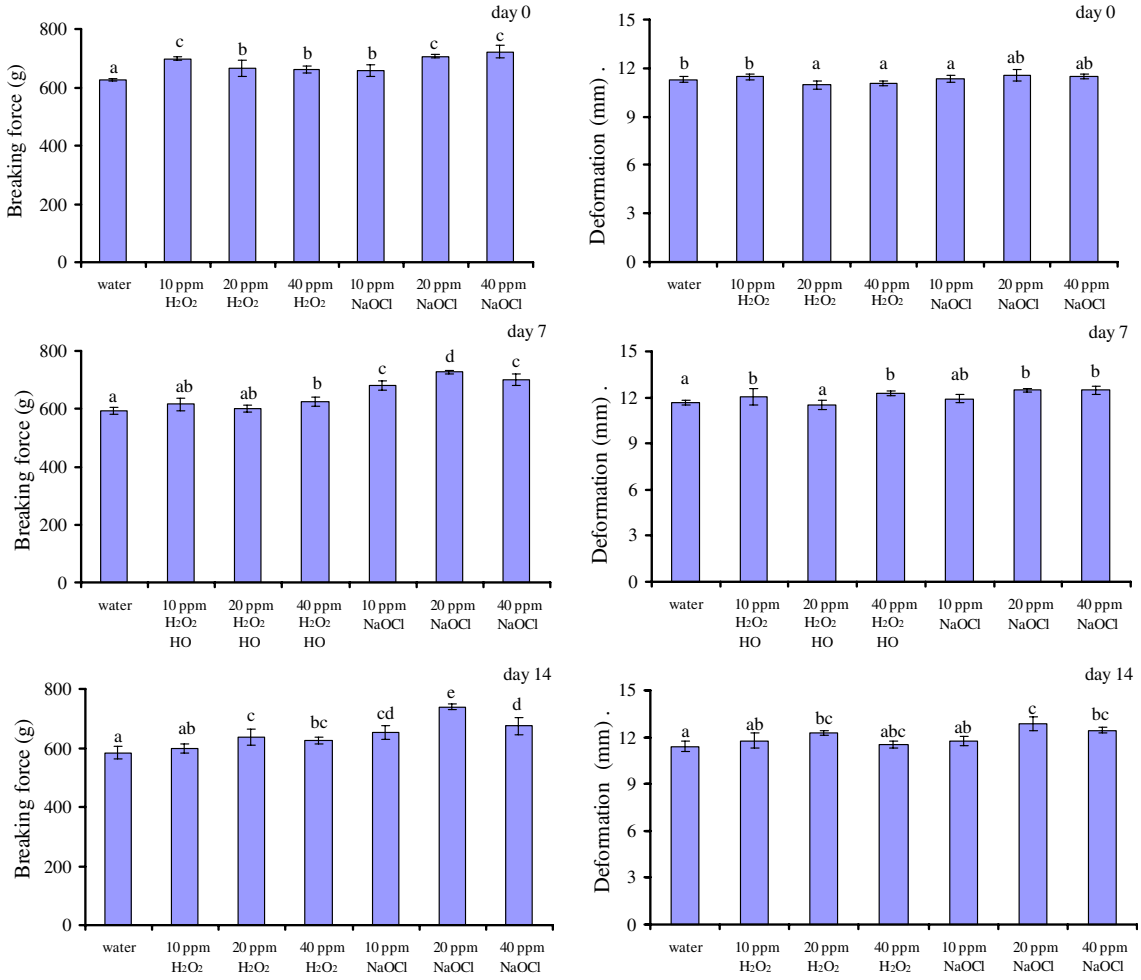

Fig. 3. Changes in breaking force and deformation of gel of surimi produced from bigeye snapper stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from five determinations.

washing increased by 11.8%. When the concentration of H_2O_2 increased up to 40 ppm, the decrease in breaking force was noticeable ($P < 0.05$). However, the breaking force of surimi with H_2O_2 washing was still higher than that of surimi with water washing ($P < 0.05$). At days 7 and 14, a greater concentration of H_2O_2 resulted in a higher breaking force of the surimi obtained. This result indicated that H_2O_2 could improve gel-forming ability of surimi to some extent. Bhoite-Solomon, Kessler-Icekson, and Shaklai (1992) reported that H_2O_2 alone could cause myosin to form disulfide-cross-linked aggregates but did not induce fragmentation of myosin. Washing with NaOCl solution at all concentrations gave a surimi with higher breaking force than did water or H_2O_2 washing. NaOCl exhibited a greater gel-strengthening effect on surimi, which was produced from the fish with a longer storage time. It was found that washing with NaOCl at 20 ppm resulted in the highest breaking force of surimi produced from fish stored for 7 and 14 days in ice. The decrease in breaking force of surimi prepared by washing with 40 ppm NaOCl might be due to the excessive denaturation of proteins induced by oxidising agents, leading to a poorer gel-forming ability. Nevertheless, washing with 20 ppm of NaOCl might cause some degree of oxidation of protein in a fashion, which resulted in increase of the protein chain length. As a consequence, the gel could be formed with a longer strand of protein filaments. NaOCl is an oxidising agent commonly used in water for food processing to reduce the microbial load (Rossoni & Gaylarde, 2000). Therefore, washing bigeye snapper mince with NaOCl solution at a concentration of 20 ppm could effectively increase breaking force of resulting surimi.

Deformation of surimi produced from bigeye snapper after various storage times in ice with different washing media is shown in Fig. 3. At day 0, H_2O_2, at the higher concentration, resulted in decreases in the deformation of surimi, compared with that of surimi prepared by water washing. No differences in the deformation of surimi produced by washing with NaOCl, at any concentrations, were observed ($P > 0.05$). Generally, the deformation of surimi prepared by H_2O_2 washing of fish

mince stored for 7 and 14 days were similar and not different from that with water washing. However, the greater deformation was noticeable when NaOCl solutions, at concentrations of 20 and 40 ppm, were used as the washing medium ($P < 0.05$), in comparison with surimi prepared by water washing, particularly for the fish kept in ice for 7 and 14 days. Thus, the use of NaOCl at 20 ppm as washing medium was demonstrated as a promising way to improve both breaking force and deformation of resulting surimi.

3.4. Effect on surface hydrophobicity

Surface hydrophobicities of NAM extracted from mince washed with water and 20 ppm NaOCl solution was compared (Fig. 4). Generally, mince washed with NaOCl solution had a higher surface hydrophobicity than that washed with water ($P < 0.05$). Our results indicated that washing mince with NaOCl solution might induce conformational changes in protein to some extent, as evidenced by the increase in surface hydrophobicity. ANS, a fluorescence probe, has been found to bind to hydrophobic amino acids containing an aromatic ring, such as phenylalanine and tryptophan, and can be used to indicate the conformational changes occurring in the protein. With increasing time of iced storage, surface hydrophobicity of NAM increased in both minces washed with water and with NaOCl solution ($P < 0.05$). Benjakul et al. (1997) found that surface hydrophobicity of natural actomyosin of Pacific whiting increased by 56% after 2 days of iced storage and remained constant during the next 6 days. Roura, Saavedra, Truco, and Crupkin (1992) reported that the surface hydrophobicity of hake actomyosin increased during iced storage, particularly during the first 3 days. The increase in surface hydrophobicity at day 7 was possibly caused by the exposure of hydrophobic groups of the protein molecule. During the denaturation or degradation processes, the hydrophobic and hydrogen bonds buried in the interior of the protein molecule become exposed and broken from their native arrangement with consequent conformational changes in coiled or helical sections of the peptide chain (Morawezt, 1972). Slight decrease in surface hydrophobicity observed at day 14 might be due to the association of hydrophobic portion via hydrophobic interaction, leading to less exposure of hydrophobic groups.

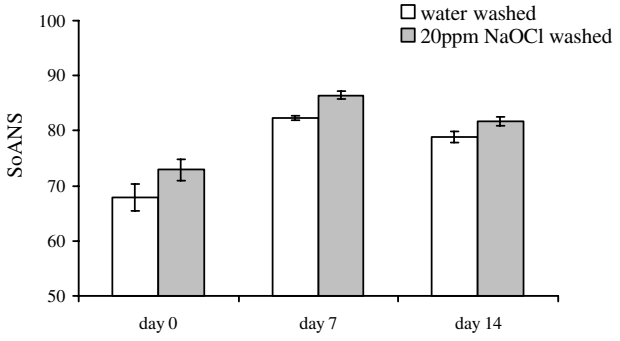

Fig. 4. Surface hydrophobicity of NAM extracted from bigeye snapper mince stored in ice for different times and washed with water or 20 ppm NaOCl. Bars indicate the standard deviation from three determinations.

3.5. Effect on total sulfhydryl group and disulfide bond contents

Total sulfhydryl group content of NAM extracted from mince washed with water and 20 ppm NaOCl is shown in Fig. 5. NAM of mince washed with 20 ppm NaOCl had a lower sulfhydryl group content than that of mince washed with water ($P < 0.05$). A decrease in total sulfhydryl group content was reported to be due to the formation of disulfide bonds through oxidation of sulfhydryl groups or disulfide interchanges (Hayakawa & Nakai, 1985). Benjakul et al. (1997) found that total sulfhydryl group content of Pacific whiting actomyosin increased slightly after 2 days of iced storage, followed by a gradual decrease for up to 8 days. NAM extracted from mince washed with water had a lower disulfide bond content than that washed with 20 ppm NaOCl solution ($P < 0.05$). This was in accordance with the lower sulfhydryl group content remaining in the sample washed with

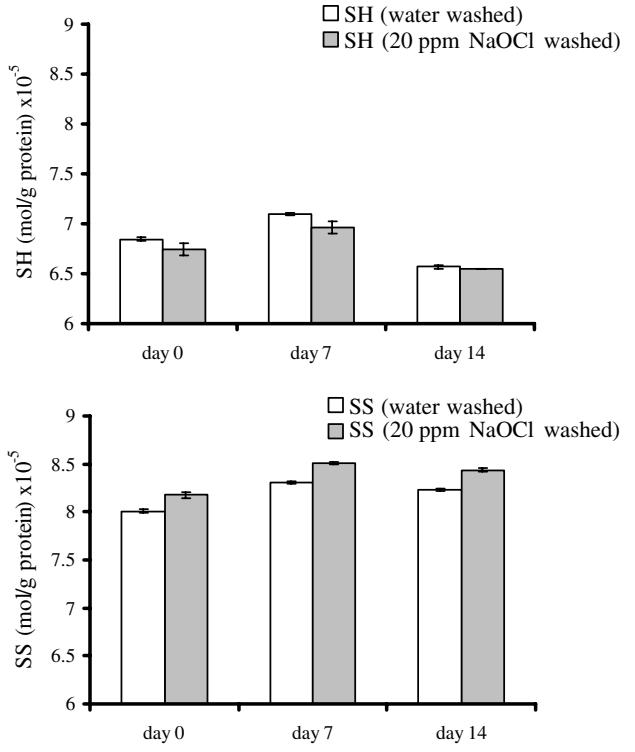

Fig. 5. Total sulfhydryl group and disulfide bond contents of NAM extracted from bigeye snapper mince stored in ice for different times and washed with water or 20 ppm NaOCl. Bars indicate the standard deviation from three determinations.

NaOCl. Therefore, washing mince with NaOCl solution might result in the formation of disulfide bonds. Lanier (2000) reported that the disulfide bond is the only covalent cross-link found naturally in proteins. An intermolecular disulfide bond is formed by the oxidation of two cysteine molecules on neighbouring protein chains (Lanier, 2000). The oxidation of protein might be associated with the improved gel property of mince washed with NaOCl (Fig 3). This was probably due to the partial cross-linking of hydrolysed protein molecules induced by oxidising agent, resulting in the gel network formation with larger strands.

3.6. Effect on ATPase activities

During ice storage for 14 days, no marked differences in Ca^{2+}-ATPase, Mg^{2+}–Ca^{2+}-ATPase and Mg^{2+}-ATPase activities of NAM extracted between minces washed with water and 20 ppm NaOCl solution were observed ($P > 0.05$) (Fig. 6). Also, no changes in any activities, except higher Mg^{2+}–EGTA-ATPase, were noticeable with increasing storage time. Generally, Ca^{2+}-ATPase can be used as an indicator of the integrity of the myosin molecule. Mg^{2+}–Ca^{2+}-ATPase activity can be used as an indicator of the integrity of the actin–myosin complex (Benjakul et al., 1997). Mg^{2+}-ATPase has been used to indicate the integrity of actin (Azuma & Konno, 1998). From the results, higher Mg^{2+}–EGTA-ATPase activity of NAM of mince washed with 20 ppm NaOCl was observed than that of mince washed with water ($P < 0.05$). Additionally, increase in Mg^{2+}–EGTA-ATPase was found in both minces when the storage time was increased for up to 7 days ($P < 0.05$). This result suggested that NaOCl might affect the changes in troponin–tropomyosin complex. Benjakul et al. (1997) found that the increase in Mg^{2+}–EGTA-ATPase of Pacific whiting NAM was concomitant with the loss in Ca^{2+}-sensitivity during the extended iced storage.

3.7. Effect on protein pattern

Similar protein patterns were observed in mince washed with either water or NaOCl under reducing conditions, suggesting that negligible proteolytic degradation occurred throughout storage (Fig. 7). However, when the mince was washed with NaOCl solution, a decreased MHC band intensity was observed under non-reducing conditions when compared with that of mince washed with water, at all storage times studied. Oxidation increased the formation of numerous high-molecular-weight protein bands, which appeared as smears or dark stains at the top of the separating gel (Fig. 7). The decrease in MHC of mince washed with NaOCl was coincidental with the increase in disulfide bonds and decrease in sulfhydryl group content (Fig. 6). However, under reducing conditions, no differences in band intensity of MHC were found between minces washed with the two washing media. This result indicated that

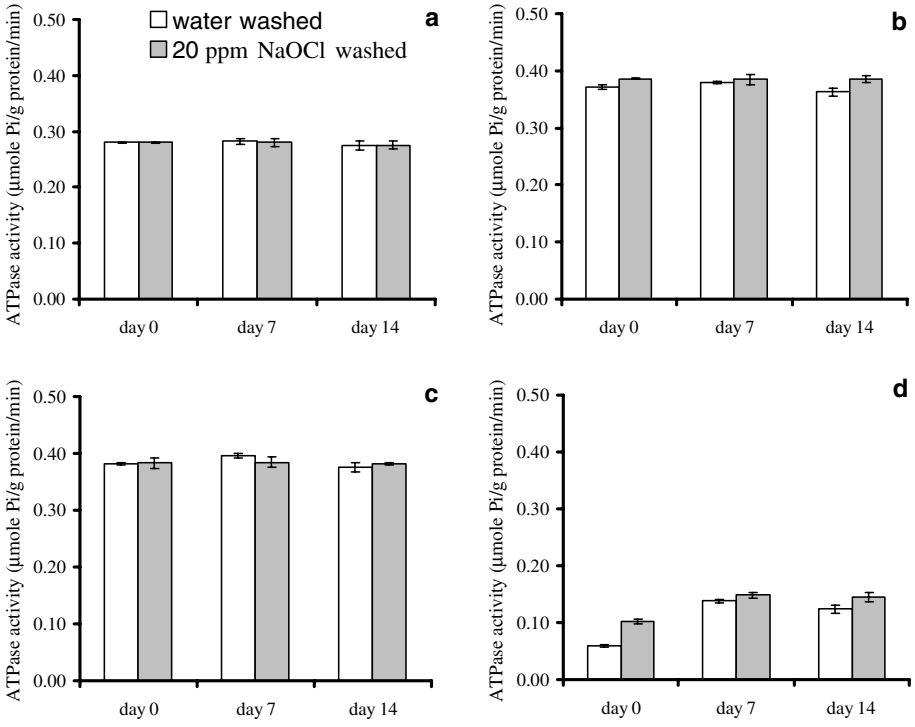

Fig. 6. Ca^{2+}-ATPase (a), Mg^{2+}-ATPase (b), Mg^{2+}–Ca^{2+}-ATPase (c) and Mg^{2+}–EGTA-ATPase (d) activities of NAM extracted from bigeye snapper mince stored in ice for different times and washed with water or 20 ppm NaOCl. Bars indicate the standard deviation from three determinations.

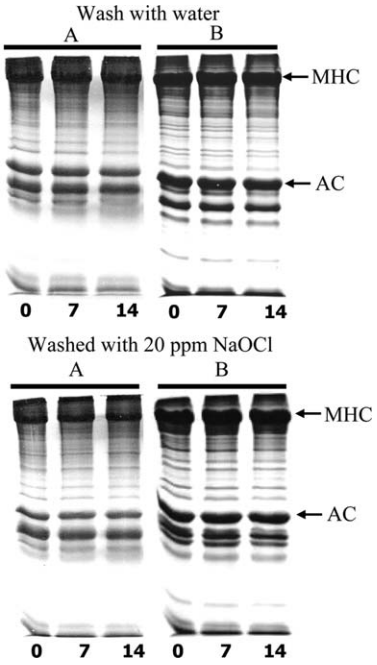

Fig. 7. SDS–PAGE pattern of bigeye snapper mince stored in ice for different times and washed with water or 20 ppm NaOCl. Numbers designate the storage time (days): (A) non-reducing; (B) reducing.

the oxidising agent might cause the oxidation of protein, especially MHC, via disulfide bond formation. The cleavage of disulfide bonds under reducing condition could split the large aggregate into lower-apparent-MW proteins. Liu and Xiong (2000) reported that chicken breast myosin, after incubation with the oxidants ($FeCl_3/H_2O_2$/ascorbate) for 24 h, had no MHC band retained. From the result, the cross-linking of MHC, with the appropriate type and concentration of oxidising agent in the wash water, might be associated with increase in gel-forming ability. From the safety point of view, the solutions of both oxidising agents were used at concentrations less than 40 ppm. Chlorine hydrolyses very rapidly in water (US EPA, 1989). So far, no conclusion on the carcinogenicity and on the developmental/reproductive toxicity of chlorine can be drawn from the limited information available for human and animal studies (US EPA, 1989). Hydrogen peroxide has a short half-life in the environment and lack of any residues of toxicological concern. Thus, the residue of both compounds in washed mince would be negligible owing to their instability. Additionally, the washing media were removed from the minces, leading to the lower amount of both compounds remaining.

4. Conclusion

Oxidising agent washing directly affected the physicochemical properties of muscle protein and gel strength of bigeye snapper surimi. Generally, 20 ppm NaOCl was the most appropriate washing medium in term of gel property improvement, especially for low-quality fish.

Acknowledgements

Authors thank Prince of Songkla University for the financial support and the scholarship for Suttirug Phatcharat.

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