Chapter 3

Results and Discussion

1. Effect of washing with various oxidizing agents on chemical composition, physicochemical properties and gel forming ability of fish mince

1.1 Changes in pH

Mince produced from bigeye snapper, threadfin bream and starry triggerfish stored in ice for different times and washed with different washing media showed the varying pHs (Figure 7). At day 0 of storage, mince washed with all media used had the similar pH. However, washing mince from bigeye snapper caused the marked decrease in pH, especially for the sample stored for a longer time ($P<0.05$). At day 14, pHs of mince from bigeye snapper washed with NaOCl were greater than those of mince washed with H_2O_2. The result suggested that those oxidizing agents might induce the cross-linking of proteins 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. From the result, the pH of threadfin bream and starry triggerfish mince and mince washed with H_2O_2 or NaOCl increased when the storage times increased. However, washing with H_2O_2 and NaOCl resulted in the decreased pH with 0, 7 and 14 day for threadfin bream, 7 and 14 day for bigeye snapper and 14 day for starry triggerfish, when compared with unwashed mince. Generally, the pH of muscle increased when the storage time increased. This was coincidental with the increase in total volatile bases, indicating the decomposition of muscle proteins (Benjakul *et al.*, 2002). Therefore, washing threadfin bream and starry triggerfish mince might remove total volatile bases to some extent.

Nevertheless, no marked differences in pH were observed between mince washed with the same washing medium at different concentrations used.

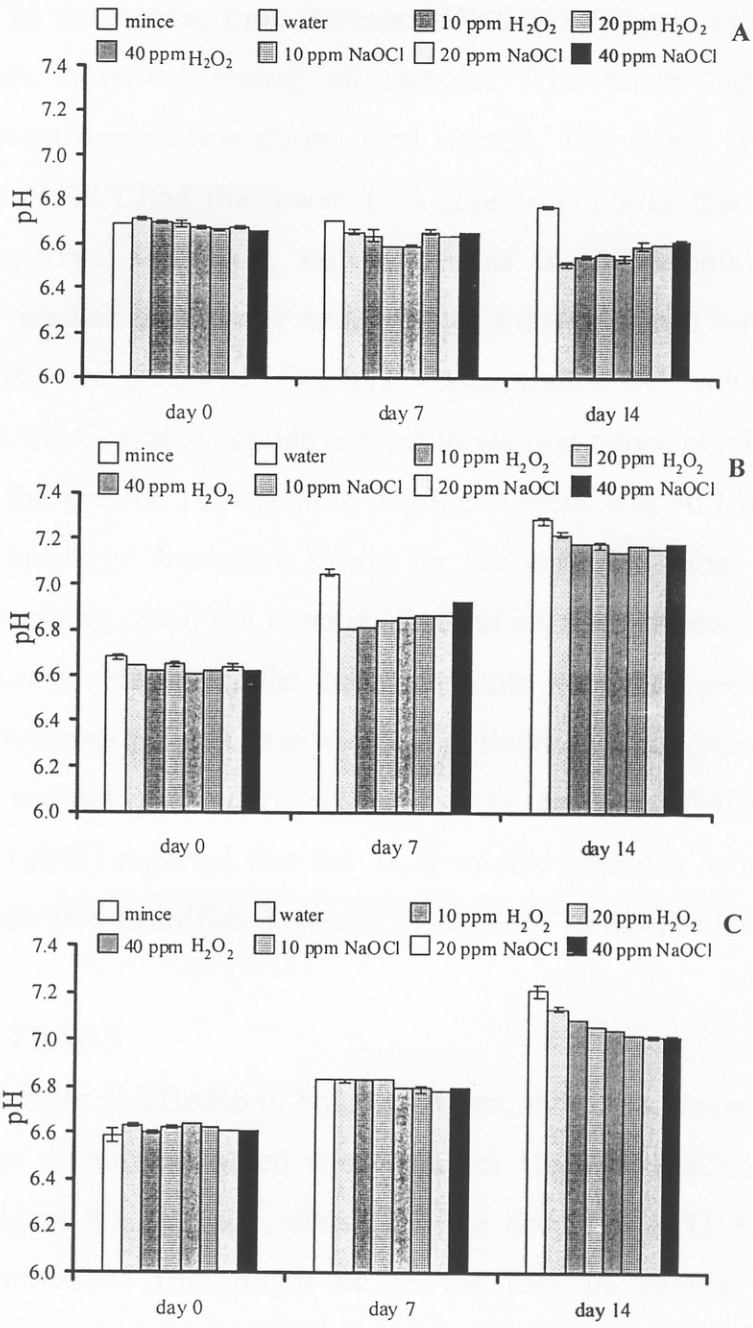

Figure 7 Changes in pH of bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from triplicate determinations.

1.2 Changes in TCA-soluble peptides

TCA-soluble peptides of bigeye snapper mince washed with all media used increased as the storage time increased ($P<0.05$). (Figure 8) No marked differenced were observed among all samples. The result indicated that proteins underwent degradation during iced storage. The mince washed with water, H_2O_2 or NaOCl had the lower TCA-soluble peptides than unwashed mince in all species. Generally, no differences in TCA-soluble peptides between mince washed with water and those of mince washed with H_2O_2 or NaOCl solutions were observed ($P>0.05$). Concentration of oxidizing agents had no effect on TCA-soluble peptide content in washed mince obtained. It was noticeable that the greater TCA-soluble peptide content was still observed in washed mince produced from fish stored for the extended time. The result suggested that washing could not remove all small peptides produced from the degradation process. However, the large amounts of small peptides were leached out by washing process as evidenced by the marked decrease in TCA-soluble peptide content, particularly when 14 days ice stored fish were used. Benjakul *et al.* (2003) reported that the TCA-soluble peptides in both whole and headed/eviscerated lizardfish increased throughout 15 days of iced storage.

1.3 Changes in TBARS

Changes in TBARS of bigeye snapper, threadfin bream and starry triggerfish mince or mince washed with water or H_2O_2 or NaOCl solutions are shown in Figure 9. Generally, mince had the decrease in TBARS as the storage time increased. This might be to the loss in volatile oxidative compounds during extended storage time. However, no changes were found in bigeye snapper mince. Washing mince with NaOCl generally caused the increased TBARS in bigeye snapper at all concentrations (about 14 to 17 mg malonadehyde/kg muscle). This might be due to the oxidizing effect of NaOCl. As a consequence, lipid oxidation was enhanced in presence of oxidizing agent.

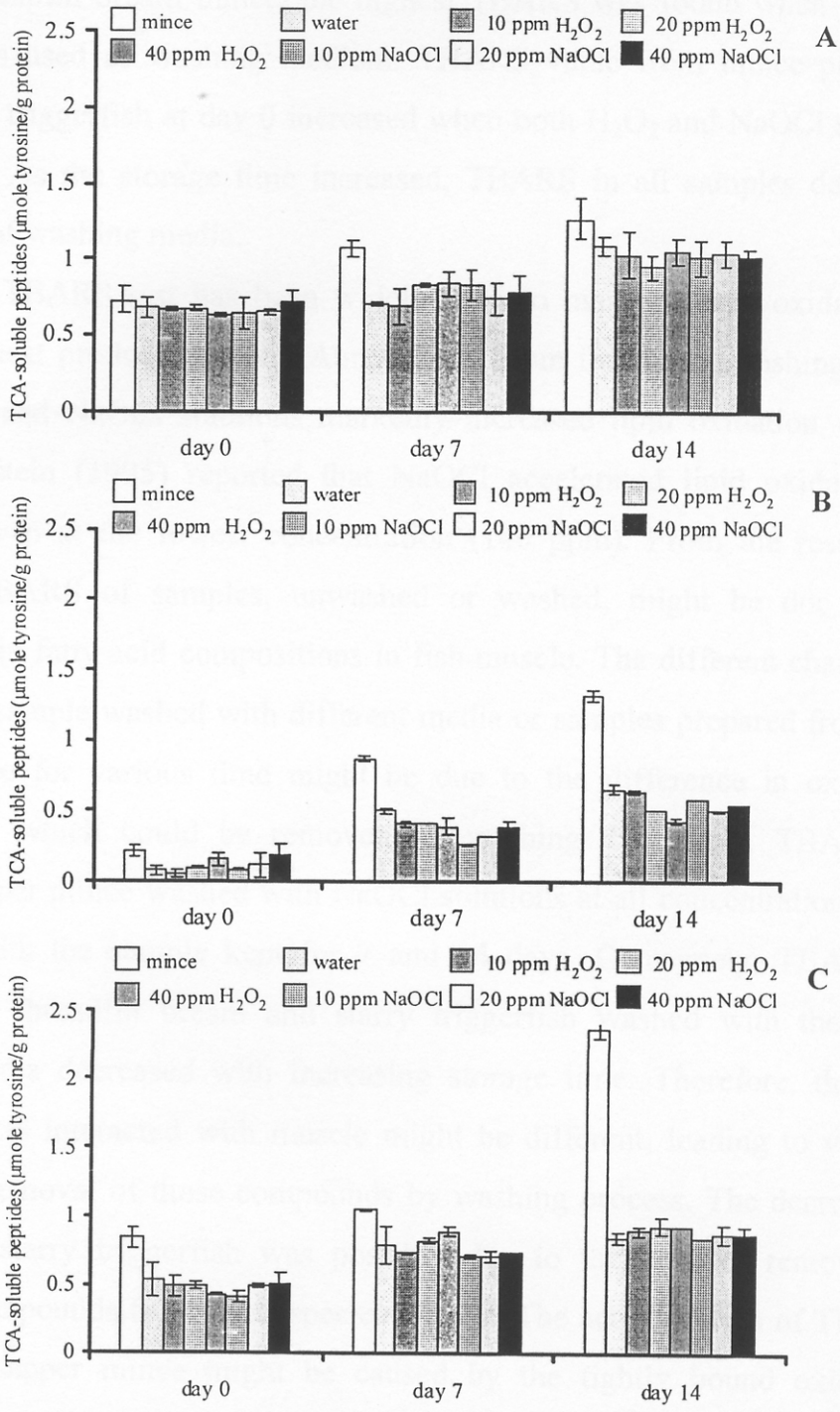

Figure 8 Changes in TCA-soluble peptides in bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from triplicate determinations.

For the threadfin bream mince, the highest TBARS was found when 40 ppm NaOCl was used as washing medium. TBARS value from mince produced from starry triggerfish at day 0 increased when both H_2O_2 and NaOCl solution were used. As the storage time increased, TBARS in all samples decreased regardless of washing media.

The TBARS test has been widely used to measure lipid oxidation in meat and meat products (Jo and Ahn, 2000). From the result, washing mince with H_2O_2 and NaOCl solutions markedly increased lipid oxidation. Hwang and Regenstein (1995) reported that NaOCl accelerated lipid oxidation of mackerel even at the lowest concentration (140 ppm). From the result, the different TBARS of samples, unwashed or washed, might be due to the differences in fatty acid compositions in fish muscle. The different changes in TBARS of sample washed with different media or samples prepared from fish stored in ice for various time might be due to the difference in oxidation compounds, which could be removed by washing differently. TBARS of bigeye snapper mince washed with NaOCl solutions at all concentrations used increased with the sample kept for 7 and 14 days. Conversely, TBARS of mince from threadfin bream and starry triggerfish washed with the same washing media decreased with increasing storage time. Therefore, the way those products interacted with muscle might be different, leading to varying efficacy in removal of those compounds by washing process. The decrease in TBARS of starry triggerfish was possibly due to the ease of removal of oxidative compounds from these species muscle. The accumulation of TBARS in bigeye snapper mince might be caused by the tightly bound oxidation products to the muscle, leading to the difficulty in leaching out those compounds. Poli *et al.* (2001) found that malonaldehyde level in the muscle of sea bass increased from 0.04 to 0.12 mg/kg when fish were maintained at 4°C for 7 days and from 0.09 to 0.19 mg/kg for fish kept at for 1°C 10 days with ice covering. However, Papadopoulos *et al.* (2003) found that malonaldehyde of

whole ungutted sea bass increased from 1.52 to 4.48 mg/kg for fish stored in ice for 16 days. Namulema *et al.* (1999) found that malonaldehyde of Nile perch increased from 0.01 to 0.23 mg/kg and decreased 0.13 mg/kg for 12 weeks when fish were maintained at -13°C for 8 weeks.

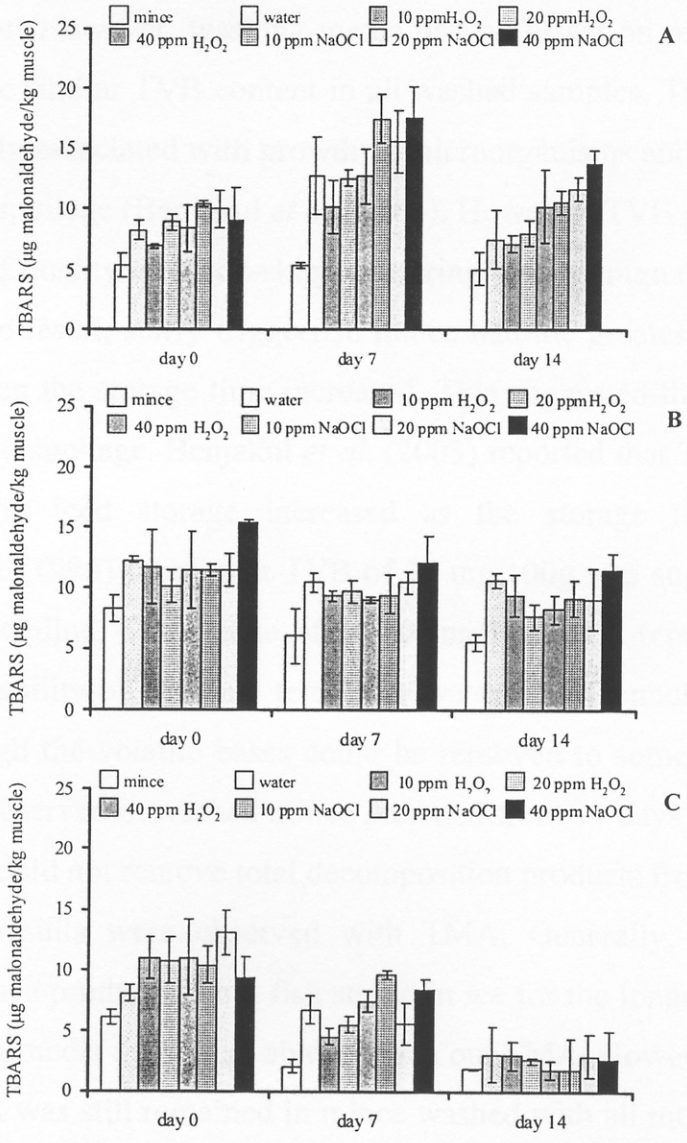

Figure 9 Changes in TBARS in bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from triplicate determinations.

1.4 Changes in TVB and TMA contents

TVB content in mince increased when storage time increased ($P<0.05$) (Figure 10). For all species, mince washed with all media, water or NaOCl or H_2O_2 solution, had the lower TVB content than unwashed mince. This suggested the removal of those decomposed volatile bases from the muscle by washing process. However, washing media had no effect on removal efficacy as shown by the similar TVB content in all washed samples. The formation of TVB is generally associated with growth of microorganisms and can be used as an indicator of spoilage (Benjakul *et al.*, 2003). However, TVB content was not a good index of quality of black-skipjack during iced storage (Mazorra *et al.*, 2000). From the result, starry triggerfish mince had the greatest TVB content, particularly when the storage time increased. This suggested the susceptibility of this species to spoilage. Benjakul *et al.* (2003) reported that TVB content of lizardfish during iced storage increased as the storage time increased. Marrakchi *et al.* (1990) found that TVB of 25 mg/100g was suggested to be a limit level for sardine. TVB value of 30-40 mg/100g was reported to be the limit for acceptability of cold and temperate water fish (Connell, 1975). From the result, though the volatile bases could be removed to some extent, higher TVB was still observed in washed mince prepared from 14 days ice stored fish. Thus washing could not remove total decomposition products from the mince.

Similar results were observed with TMA. Generally, TMA content increased in mince produced from fish stored in ice for the longer time (Figure 11). All washing media used were able to leach out TMA. However, the greater amount of TMA was still remained in mince washed with all media, especially when mince was prepared from the fish stored in ice for a longer time. However, washing mince with water or NaOCl or H_2O_2 solutions could decrease TMA in mince. The formation of TMA is generally associated with growth of microorganisms and can be used as an indicator of spoilage

(Benjakul *et al.*, 2003). Bennour *et al.* (1991) found a TMA of 5 mg/100g as the rejection value for mackerel.

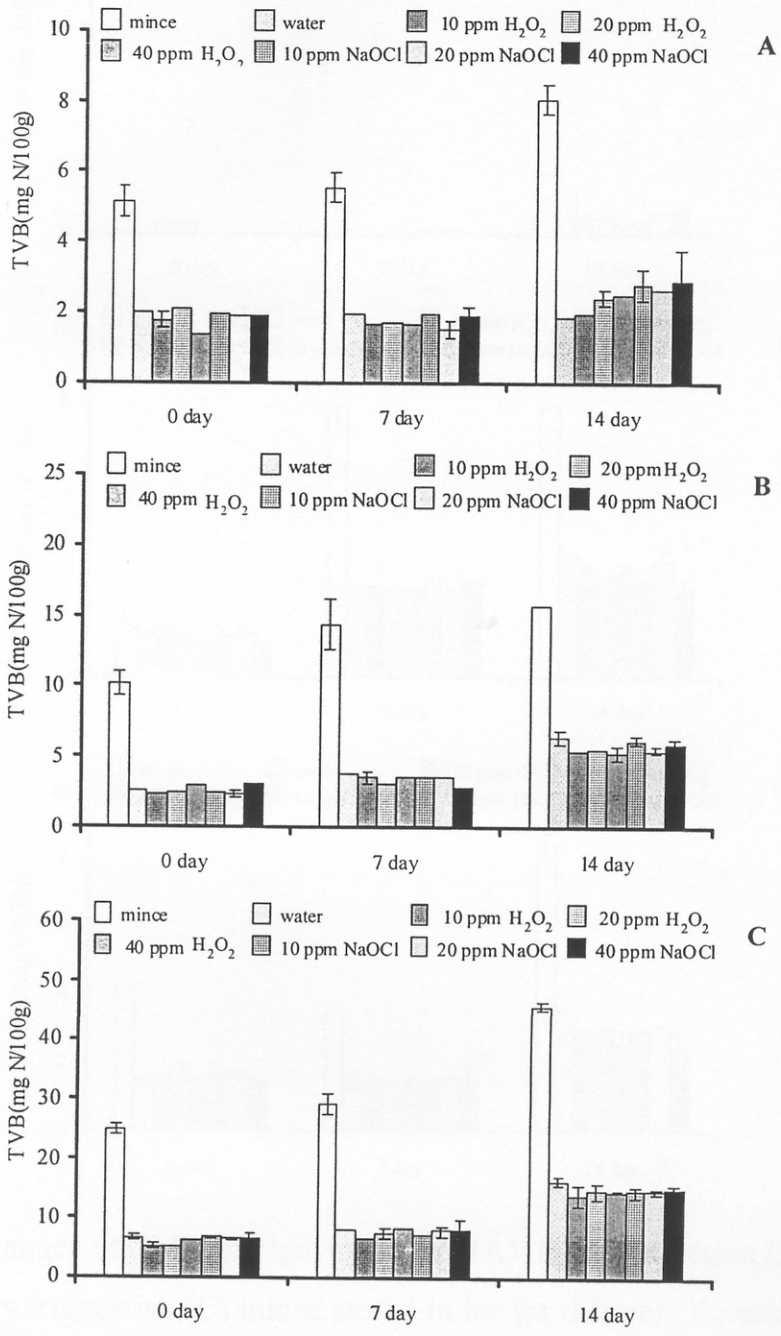

Figure 10 Changes of TVB in bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from triplicate determinations.

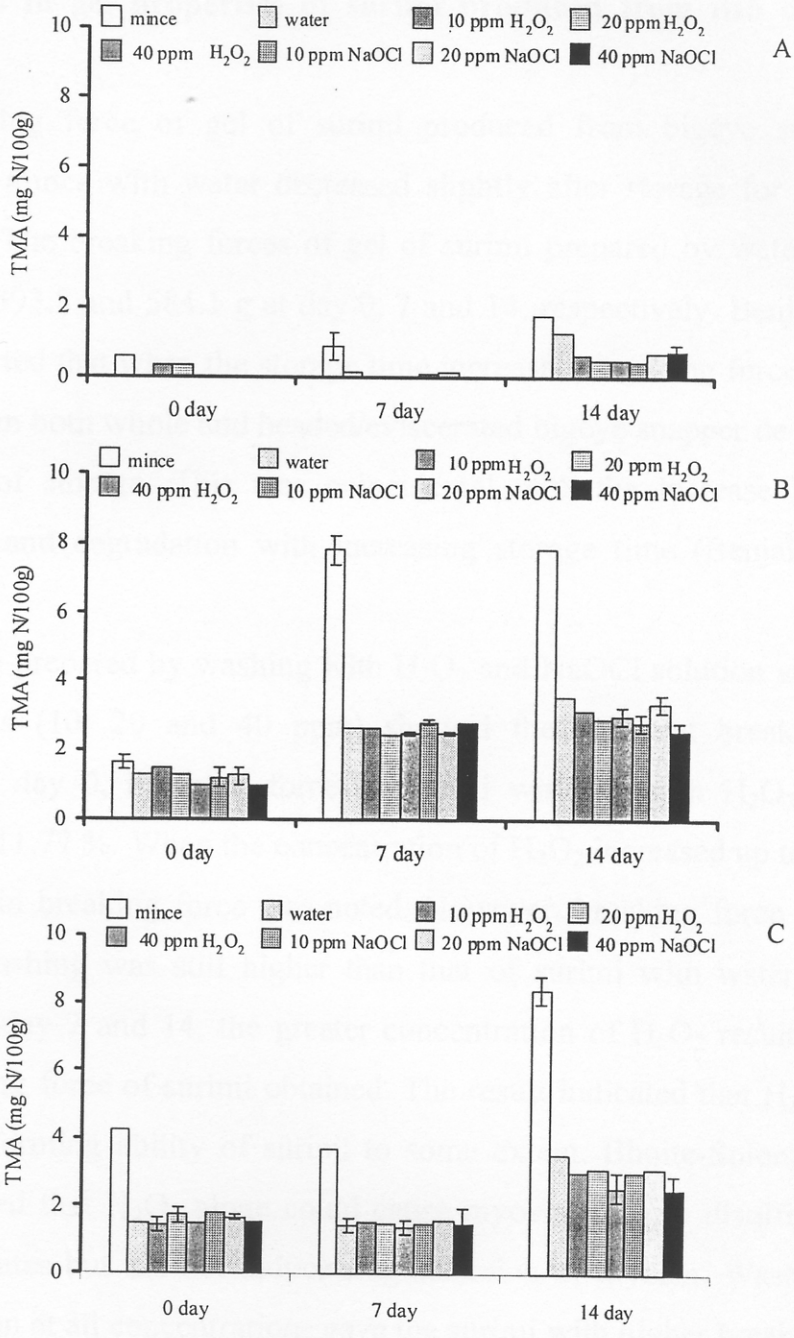

Figure 11 Changes of TMA in bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from triplicate determinations.

1.5 Changes in gel properties of surimi produced from fish during ice storage

Breaking force of gel of surimi produced from bigeye snapper by washing the mince with water decreased slightly after storage for 7-14 days (Figure 12). The breaking forces of gel 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, 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 and NaOCl solution at different concentrations (10, 20 and 40 ppm) showed the different breaking force ($P<0.05$). At day 0, breaking force of surimi with 10 ppm H_2O_2 washing increased by 11.77 %. When the concentration of H_2O_2 increased up to 40 ppm, the decrease in breaking force was noted. However, breaking force of surimi with H_2O_2 washing was still higher than that of surimi with water washing ($P<0.05$). At day 7 and 14, the greater concentration of H_2O_2 resulted in the higher breaking force of surimi obtained. The result indicated that H_2O_2 could improve gel-forming ability of surimi to some extent. Bhoite-Solomon *et al.* (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 the surimi with higher breaking force when compared with water or H_2O_2 washing. NaOCl exhibited the 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 owing to the excessive denaturation of proteins induced by

oxidizing agents, leading to the poorer gel forming ability. Nevertheless, washing with 20 ppm NaOCl might cause some degree of oxidation of protein in the fashion, which resulted in the increase in the protein chain length. As a consequence, the gel could be formed with the longer strand of protein filaments. NaOCl is shown as the oxidizing agent commonly used in water for food processing to reduce the microbial load (Rossoni and Gaylarde, 2000). Therefore, washing bigeye snapper mince with NaOCl solution at a concentration of 20 ppm could increase breaking force of resulting surimi effectively.

Deformation of surimi produced from bigeye snapper having various storage times in ice with different washing media is shown in Figure 13. H_2O_2 at the higher concentration resulted in the decreases in deformation of surimi produced from fish kept at day 0, compared with that of surimi prepared by water washing. No differences in deformation of surimi produced by washing with NaOCl at all concentrations were observed ($P>0.05$). Generally, the similar deformation of surimi prepared by H_2O_2 washing of fish mince stored for 7 and 14 days was observed and the deformation was not different from that with water washing. However, the greater deformation was noticeable when NaOCl at the concentration of 20 ppm and 40 ppm were used as the washing media ($P<0.05$), in comparison with surimi prepared by water washing. Thus, the use of NaOCl at 20 ppm as washing media was demonstrated as the promising means to improve both breaking force and deformation of resulting surimi.

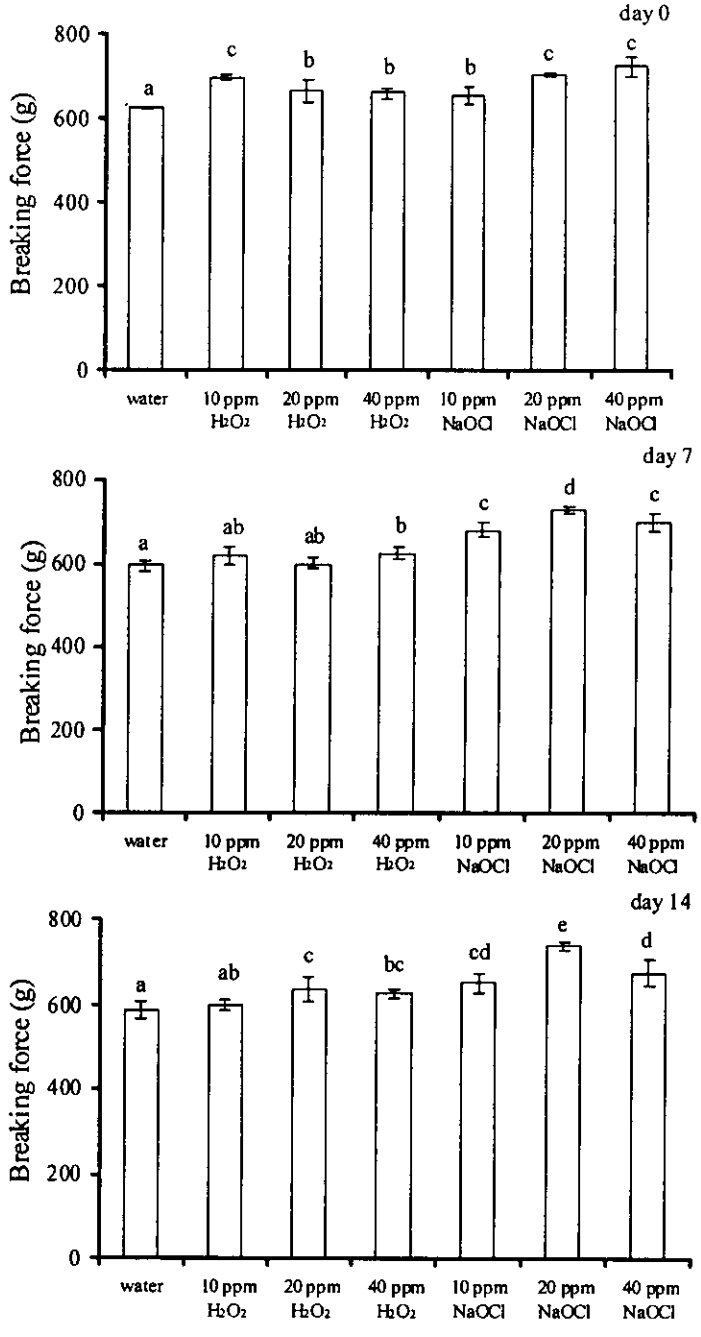

Figure 12 Changes in breaking force of gel of surimi produced from bigeye snapper stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from five determinations.

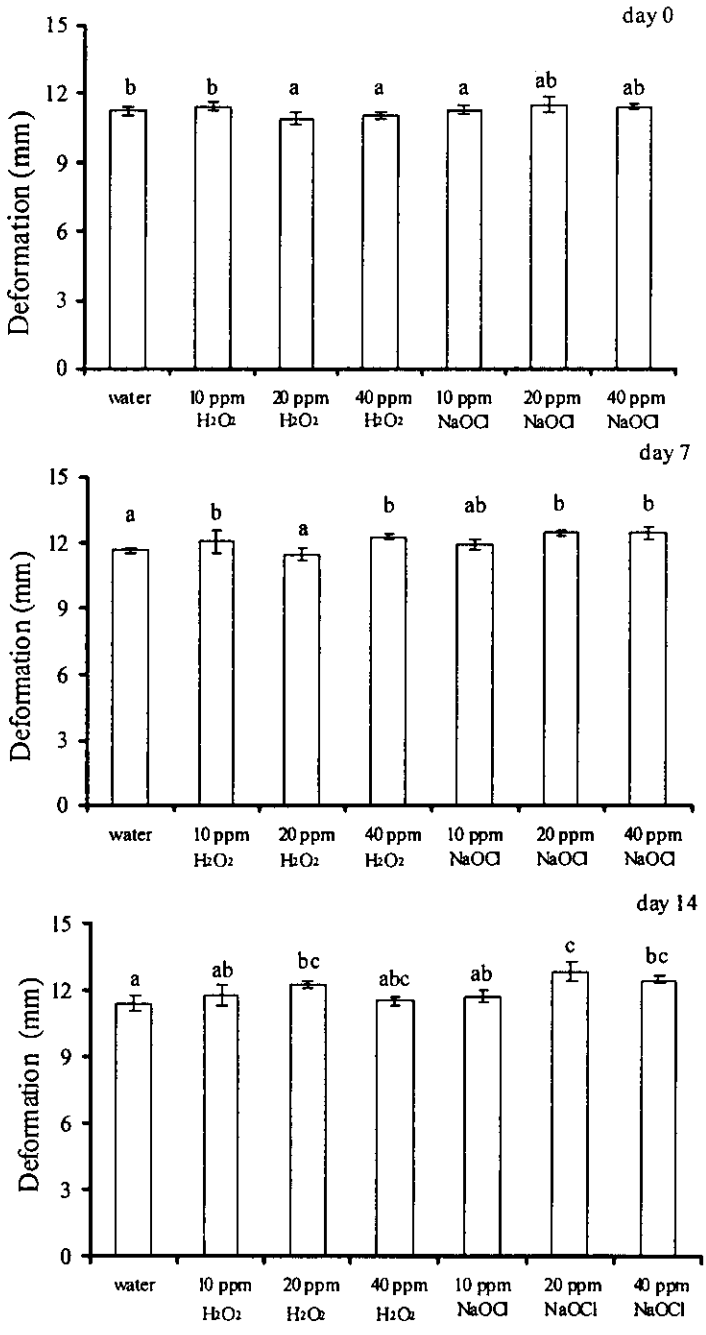

Figure 13 Changes in deformation of gel of surimi produced from bigeye snapper stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from five determinations.

Breaking force and deformation of surimi gel prepared from threadfin bream by water washing process decreased slightly after storage for 7-14 days (Figure 14). The breaking forces were 317.4, 284.9 and 254.1 g at day 0, 7 and 14, respectively. Deformations of 10.1, 9.2 and 8.8 mm were observed at day 0, 7 and 14, respectively (Figure 15). Washing with H_2O_2 generally decreased both breaking force and deformation. Greater concentration of H_2O_2 used caused the higher decrease in both breaking and deformation ($P<0.05$). However, washing with NaOCl solution resulted in the increase both in breaking force and deformation ($P<0.05$). The greater breaking force was found in mince produced from fresh fish (day 0) when 20 ppm NaOCl was used. Interestingly, breaking force and deformation increased by 77.6 % in mince prepared from 14 days ice-stored fish. However, washing mince at day 7 and 14 using NaOCl solution at a concentration of 40 ppm decreased both breaking force and deformation. The excessive amount of oxidizing agent might cause the drastic denatuation of protein, particularly by intermolecular aggregation. Those denatured proteins lost their gel-forming ability as evidenced by the lowered breaking force and deformation. From the result, washing surimi prepared from fish stored for 14 day or low quality fish with NaOCl solution at a concentration of 20 ppm could increase the gel strength of surimi.

Breaking force and deformation of surimi gel prepared from starry triggerfish by different washing media are shown in Figure 16 and 17, respectively. Breaking force of surimi gel prepared by water washing decreased from 215.3 g at day 0 to 172.1 g at day 14 and deformation decreased from 6.0 mm at day 0 to 5.6 mm at day 14. When H_2O_2 solution was used, slight increase in breaking force was found at all concentrations used at day 0, while at day 7, the breaking force increased when washed with 40 ppm H_2O_2. At day 14, higher breaking force was found when 10 and 20 ppm H_2O_2 solutions were used. Mince washed with 20 ppm NaOCl had breaking force increased by 15,

28 and 7 % when the fish stored for 0, 7 and 14 were used, respectively. From the result, it can be inferred that washing with NaOCl at suitable concentration can increase breaking force and deformation of mince even though the poor quality fish were used as the raw material. Therefore, the gel strength was influenced by the type and concentration of oxidizing agent.

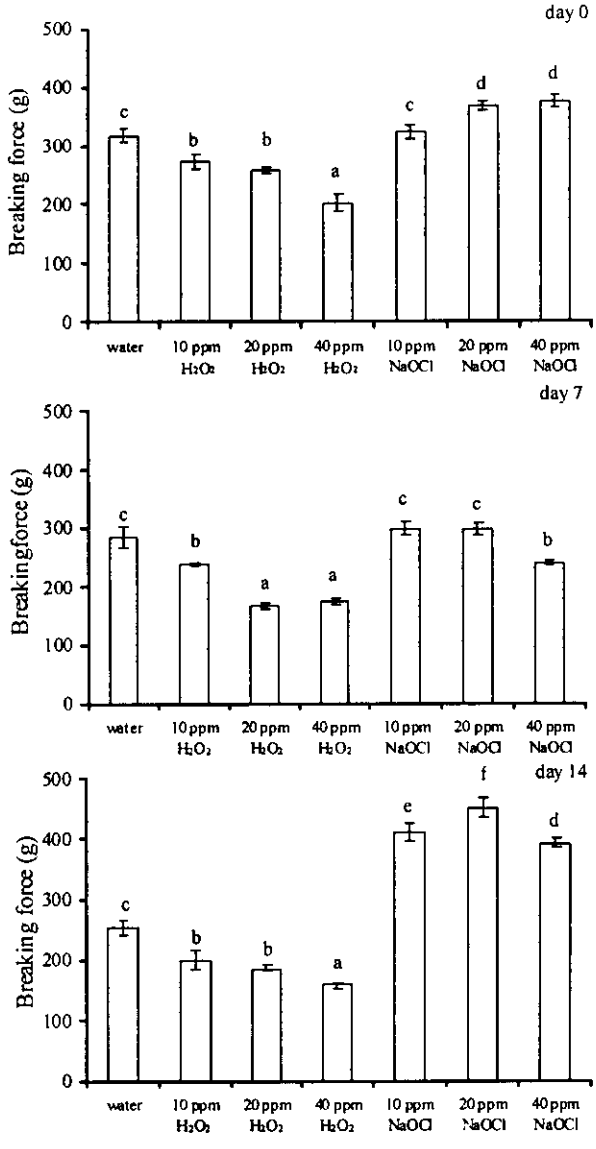

Figure 14 Changes in breaking force of gel of surimi produced from threadfin bream stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from five determinations.

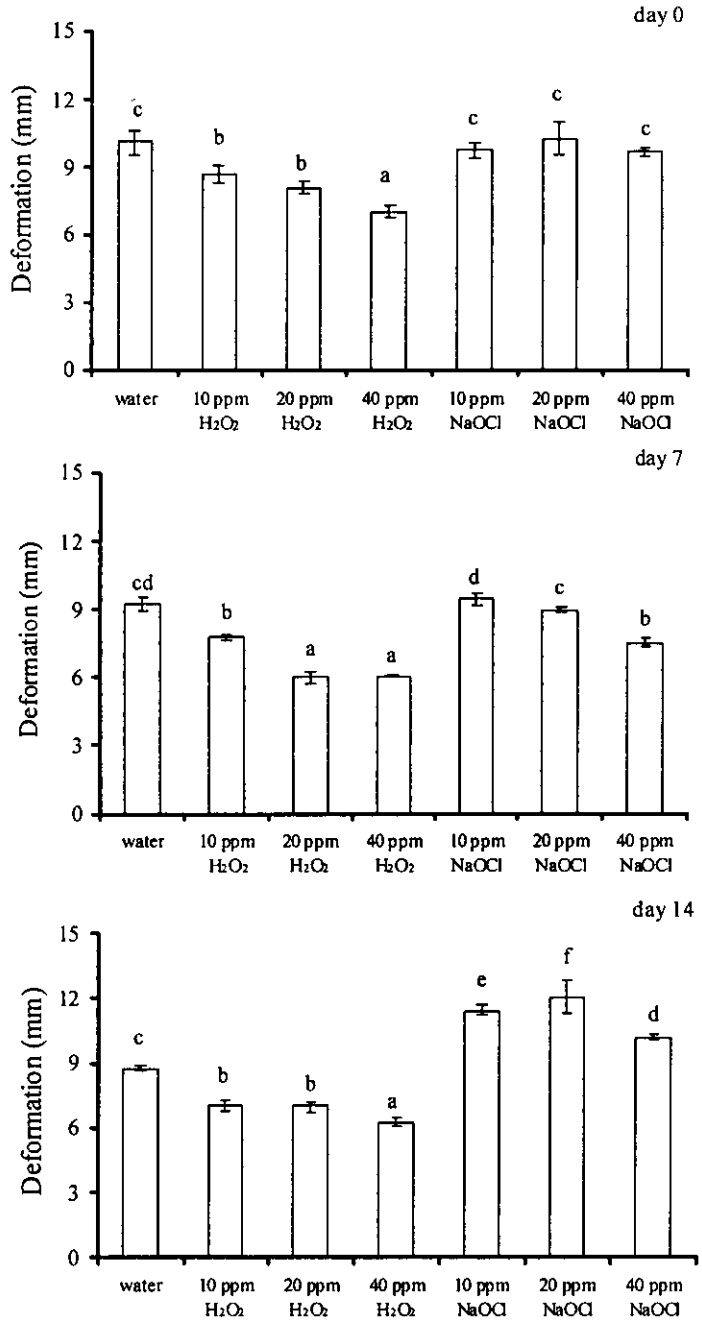

Figure 15 Changes in deformation of gel of surimi produced from threadfin bream stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from five determinations.

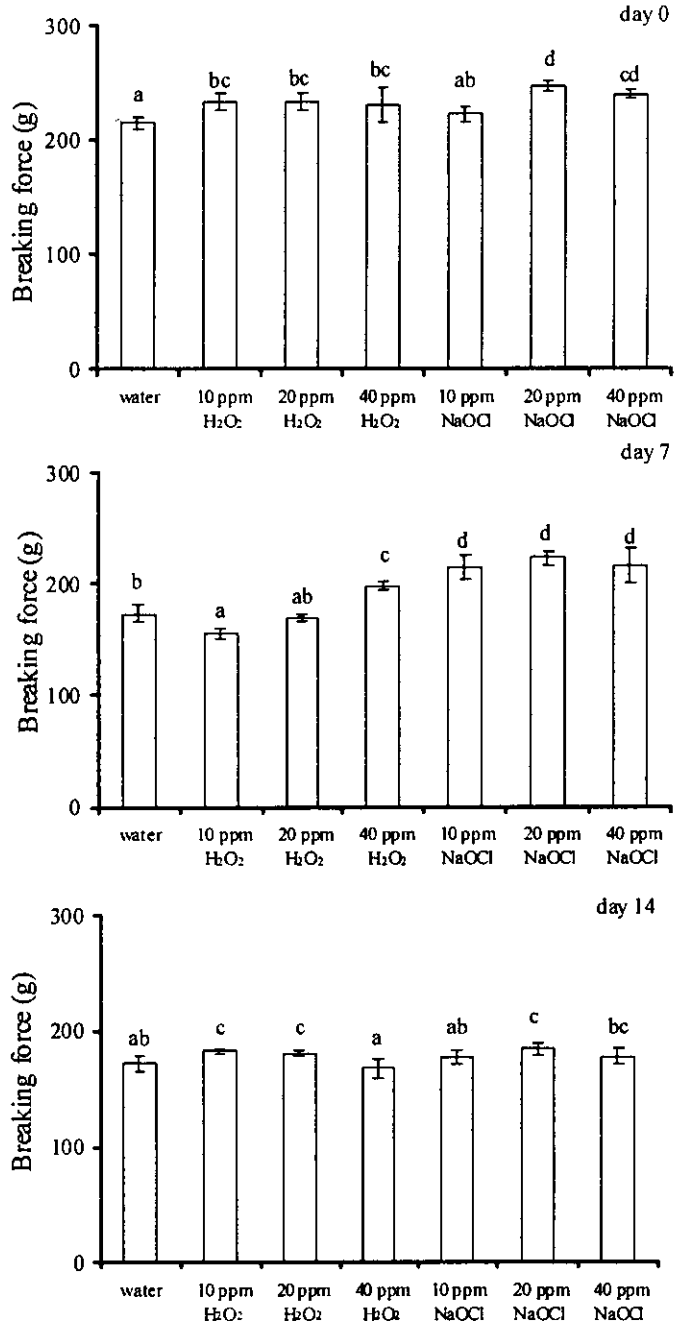

Figure 16 Changes in breaking force of gel of surimi produced from starry triggerfish stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from five determinations.

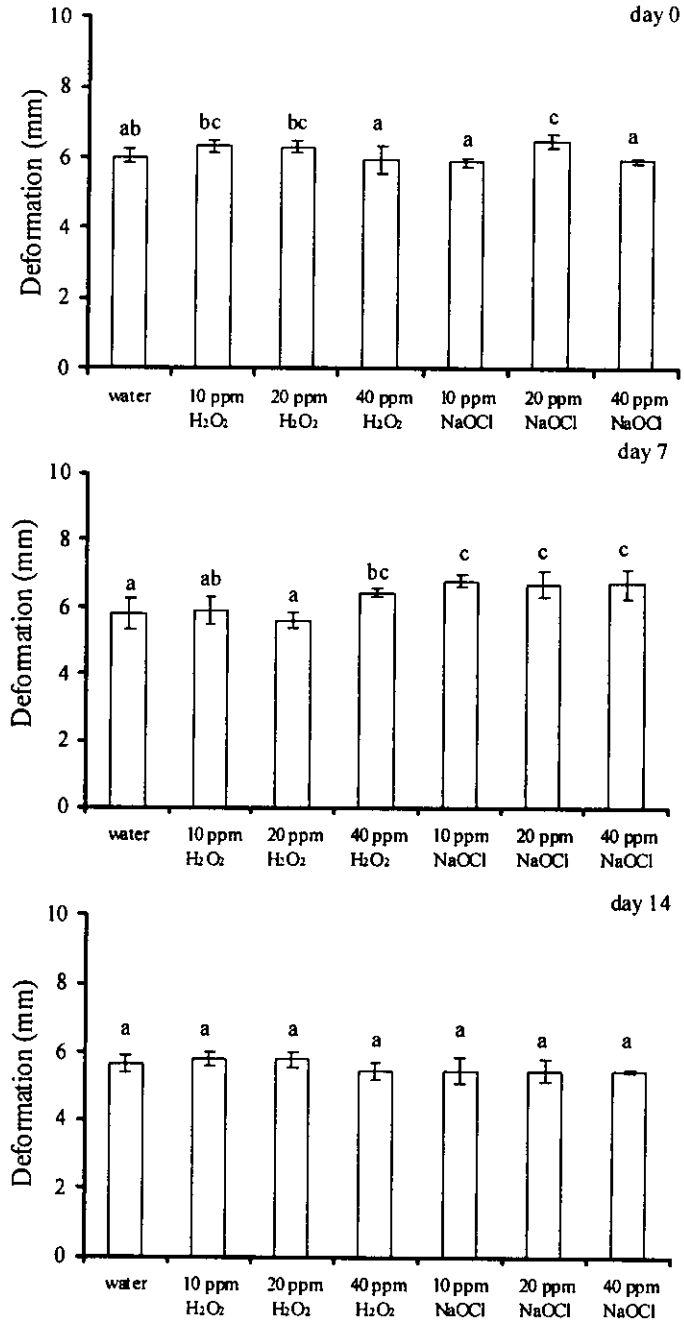

Figure 17 Changes in deformation of gel of surimi produced from starry triggerfish stored in ice for different times and washed with different washing media. Bars indicate the standard deviation from five determinations.

1.6 Changes in whiteness of surimi gel

Whiteness of surimi gels produced from bigeye snapper slightly decreased as the storage time increased regardless of washing media. Washing with H_2O_2 and NaOCl solutions decreased whiteness to some extent (Table 1). For whiteness of surimi gel produced from threadfin bream, it decreased when the concentration of NaOCl solution increased, particularly when fish stored for 0 and 7 days were used (Table 2). Similar whiteness of surimi gels from starry triggerfish was observed in comparison with that of threadfin bream (Table 3). However, the whiteness increased for the mince produced from 14 days iced stored threadfin bream, when 10 and 20 ppm NaOCl solutions were used. This might be due to the oxidation of some pigments in the fish muscle by NaOCl. However, the excessive amounts of NaOCl (40 ppm) might cause the cross-linking of pigments with muscle, leading to more pigments remained after washing process. Benjakul *et al.* (2003) reported that whiteness of lizardfish surimi gel markedly decreased as storage time increased. During iced storage, the oxidation of pigments in fish muscle, particularly myoglobin or hemoglobin, occurred. These oxidized products possibly bound tightly with muscle proteins, especially in the presence of formaldehyde and could not be removed by washing. During extended storage, blood and liquid from internal organs in whole samples could penetrate through the muscle, especially when autolysis proceeded and caused a looser muscle structure (Benjakul *et al.*, 2003). Washing mince with NaOCl or H_2O_2 at appropriate concentration might oxidize the pigments in fish muscle, resulting in the improved whiteness of surimi gel. Therefore, NaOCl at a concentration of 20 ppm was chosen as the washing medium for further study. Physicochemical properties of mince washed with 20 ppm NaOCl were studied in comparison with those of mince washed with water.

Table 1 Whiteness of surimi gels produced from bigeye snapper stored in ice for different times and washed with different washing media

washed	day 0	day 7	day 14
water	78.60±0.20 c	76.40±0.09 c	76.62±0.10 c
10 ppm H_2O_2	77.62±0.15 b	75.27±0.24 a	76.24±0.13 c
20 ppm H_2O_2	76.81±0.14 a	75.69±0.25 ab	75.69±0.23 b
40 ppm H_2O_2	77.05±0.08 a	75.23±0.32 a	75.08±0.43 a
10 ppm NaOCl	77.88±0.19 b	75.57±0.35 ab	75.11±0.19 a
20 ppm NaOCl	76.83±0.12 a	75.97±0.33 bc	74.70±0.34 a
40 ppm NaOCl	76.87±0.17 a	75.85±0.27 b	74.86±0.09 a

*Values are mean ± standard deviation (n=4). Values with the same letter in the same column are not significantly different (P>0.05).

Table 2 Whiteness of surimi gels produced from threadfin bream stored in ice for different times and washed with different washing media

washed	day 0	day 7	day 14
water	76.94±0.18 d	77.12±0.19 c	77.69±0.18 b
10 ppm H_2O_2	76.18±0.07 ab	76.76±0.23 ab	77.00±0.28 a
20 ppm H_2O_2	76.28±0.22 bc	77.03±0.14 b	77.54±0.14 b
40 ppm H_2O_2	76.44±0.12 c	77.13±0.18 c	77.58±0.17 b
10 ppm NaOCl	76.86±0.04 d	76.77±0.10 ab	78.43±0.10 c
20 ppm NaOCl	77.20±0.06 e	77.84±0.10 d	78.45±0.23 c
40 ppm NaOCl	75.99±0.07 b	76.71±0.96 a	76.88±0.09 a

*Values are mean ± standard deviation (n=4). Values with the same letter in the same column are not significantly different (P>0.05).

Table 3 Whiteness of surimi gels produced from starry triggerfish stored in ice for different times and washed with different washing media

washed	day 0	day 7	day 14
water	71.14±0.15 c	75.27±0.15 c	73.95±0.44 ab
10 ppm H_2O_2	71.21±0.20 c	75.57±0.16 c	73.61±0.47 a
20 ppm H_2O_2	70.42±0.21 b	74.28±0.28 a	73.38±0.14 a
40 ppm H_2O_2	72.91±0.03 e	75.01±0.18 bc	73.84±0.27 ab
10 ppm NaOCl	71.88±0.35 d	75.19±0.27 bc	73.85±0.24 ab
20 ppm NaOCl	70.41±0.39 ab	75.08±0.39 bc	74.66±0.87 b
40 ppm NaOCl	69.89±0.35 a	74.69±0.26 b	74.33±0.69 ab

*Values are mean ± standard deviation (n=4). Values with the same letter in the same column are not significantly different ($P>0.05$).

1.7 Changes in surface hydrophobicity

Surface hydrophobicity of natural actomyosin (NAM) extracted from mince washed with water and with 20 ppm NaOCl solution was compared (Figure 18). No differences in surface hydrophobicity of NAM from bigeye snapper washed mince were observed between both treatments when fish at day 0 was used ($P>0.05$). As the storage time increased at 7 day, surface hydrophobicity of NAM from bigeye snapper mince washed with water and with NaOCl solution increased ($P<0.05$). At day 14, a slight decrease in surface hydrophobicity was noticeable. Changes in surface hydrophobicity of NAM extracted from starry triggerfish mince washed with either water or NaOCl solution were similar with those observed in bigeye snapper washed mince. The highest surface hydrophobicity of NAM from starry triggerfish washed mince was obtained at day 7 with the subsequent decreases at day 14. For surface hydrophobicity of threadfin bream mince washed with water, no changes were observed throughout 14 days of storage. However, slight

decrease in surface hydrophobicity was found in mince washed with NaOCl solution when the storage time increased. Benjakul *et al.* (1997) found that surface hydrophobicity of NAM of Pacific whiting increased by 56 % after 2 days of iced storage and remained constant during the next 6 days. Roura *et al.* (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 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 the following conformational changes in coiled or helical sections of the peptide chain (Morawetz, 1972). Slightly decrease in surface hydrophobicity observed at day 14 might be due to the association of hydrophobic portion via hydrophobic interaction, leading to the less exposure of hydrophobic groups. For threadfin bream, the different changes in surface hydrophobicity were observed, compared with those found in bigeye snapper and starry triggerfish samples. This result suggested that protein molecules underwent conformational changes differently, possibly owing to the varying intrinsic factors determining those changes such as molecular properties, endogenous enzyme, etc. Generally, mince washed with NaOCl solution had the higher surface hydrophobicity than that washed with water ($P<0.05$). Our results indicated that washing mince with NaOCl solution might induce the conformational changes of proteins to some extent as evidenced by the increase in surface hydrophobicity. ANS, a fluorescence probe, has been found to bind to the hydrophobic amino acids containing an aromatic ring, such as phenylalanine and tryptophan, and can be used to indicate the conformational changes occurred in the protein.

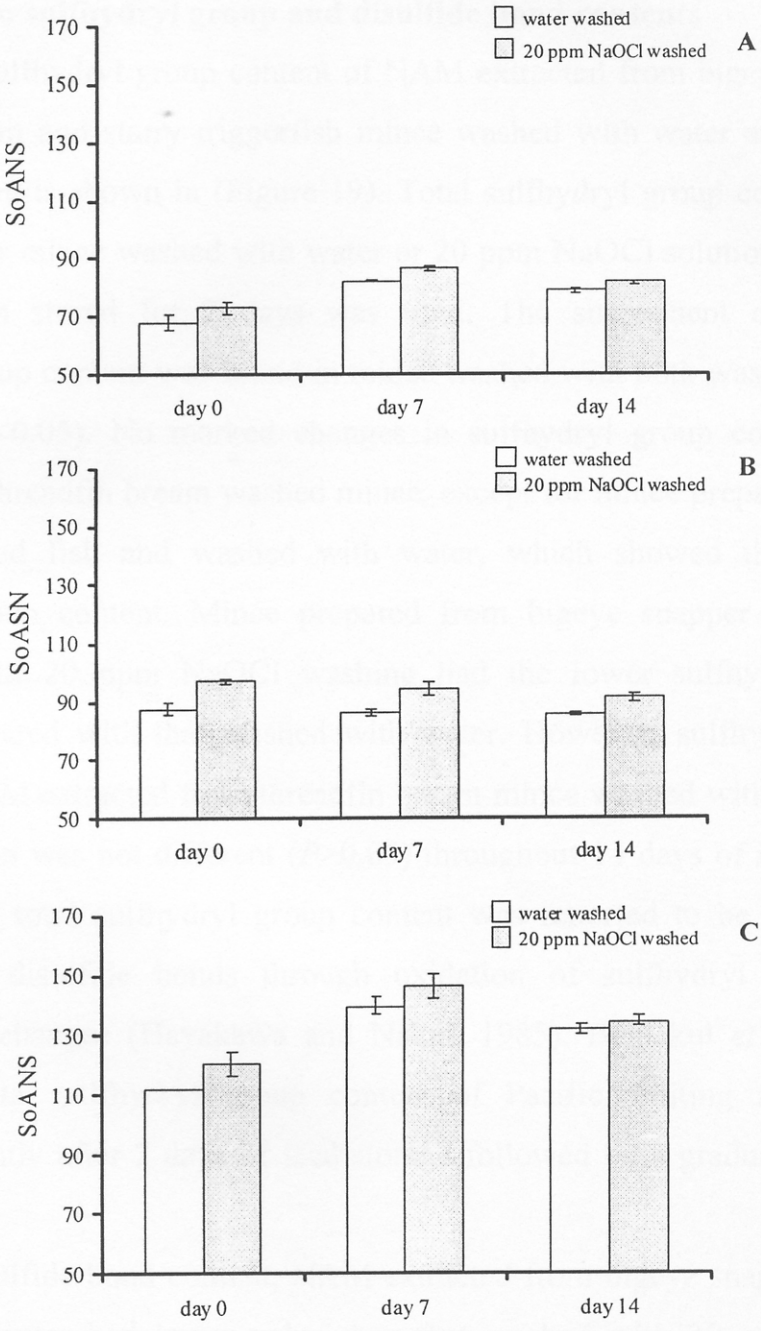

Figure 18 Surface hydrophobicity of NAM extracted from bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with water or 20 ppm NaOCl. Bars indicate the standard deviation from triplicate determinations.

1.8 Changes in sulfhydryl group and disulfide bond contents

Total sulfhydryl group content of NAM extracted from bigeye snapper, threadfin bream and starry triggerfish mince washed with water and 20 ppm NaOCl solution is shown in (Figure 19). Total sulfhydryl group content from bigeye snapper mince washed with water or 20 ppm NaOCl solution increased when the fish stored for 7 days was used. The subsequent decrease in sulfhydryl group content was found in mince washed with both washing media at day 14 ($P<0.05$). No marked changes in sulfhydryl group content were noticeable in threadfin bream washed mince, except for mince prepared from 7 days ice stored fish and washed with water, which showed the lowered sulfhydryl group content. Mince prepared from bigeye snapper and starry triggerfish with 20 ppm NaOCl washing had the lower sulfhydryl group content, compared with that washed with water. However, sulfhydryl group content of NAM extracted from threadfin bream mince washed with water and NaOCl solution was not different ($P>0.05$) throughout 14 days of ice storage. 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 and 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 up to 8 days.

For disulfide bond content, NAM extracted from bigeye snapper mince washed with water had lower value than that washed with 20 ppm NaOCl solution ($P<0.05$). This was in accordance with the lower sulfhydryl group content remained in sample washed with NaOCl. From the result, disulfide bond content increased at day 7 and 14, suggesting the increased oxidation of sulfhydyl groups during extended storage. No differences in disulfide bond content were found in threadfin bream and starry triggerfish minces throughout the storage, regardless of washing media. It was noted that the decrease in

sulfhydryl group content in starry triggerfish was found when washed with NaOCl solution. Nevertheless, no changes in disulfide bond were observed. NaOCl might induce the conformational changes of proteins in this species in the fashion which sulfhydryl groups were buried inside, but it did not induce the oxidation of sulfhydryl groups.

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

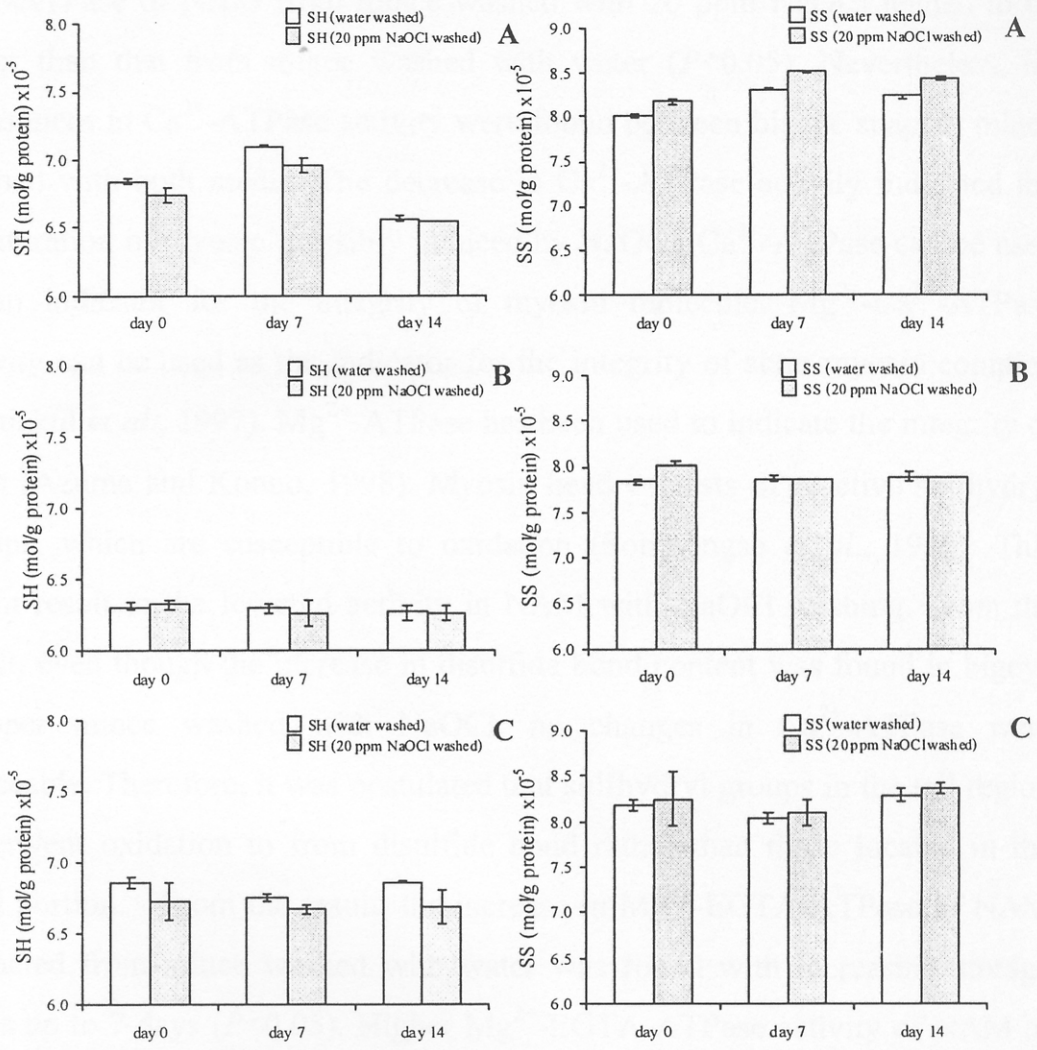

Figure19 Total sulfhydryl group and disulfide bond contents of NAM extracted from bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with water or 20 ppm NaOCl. Bars indicate the standard deviation from triplicate determinations.

1.9 Changes in ATPase activities

During iced storage of 14 days, no marked changes in Ca^{2+}-ATPase, Mg^{2+}-Ca^{2+}-ATPase and Mg^{2+}-ATPase activity of NAM extracted from minces from all three species washed with water or with 20 ppm NaOCl solution were

observed (P>0.05) (Figure 20). For threadfin bream and starry triggerfish, Ca^{2+}-ATPase of NAM from mince washed with 20 ppm NaOCl tended to be lower than that from mince washed with water (P<0.05). Nevertheless, no differences in Ca^{2+}-ATPase activity were found between bigeye snapper mince washed with both media. The decrease in Ca^{2+}-ATPase activity indicated the denaturation of myosin, possibly induced by NaOCl. Ca^{2+}-ATPase can be used as an indicator for the integrity of myosin molecule. Mg^{2+}-Ca^{2+}-ATPase activity can be used as the indicator for the integrity of actin-myosin complex (Benjakul *et al.*, 1997). Mg^{2+}-ATPase has been used to indicate the integrity of actin (Azuma and Konno, 1998). Myosin head consists of reactive sulfhydryl groups, which are susceptible to oxidation (Sompongse *et al.*, 1996). This might result in the lowered activity in NAM with NaOCl washing. From the result, even though the increase in disulfide bond content was found in bigeye snapper mince washed with NaOCl, no changes in Ca^{2+}-ATPase were noticeable. Therefore, it was postulated that sulfhydryl groups in the tail region underwent oxidation to from disulfide bond rather than those located in the head portion. From the result, the increase in Mg^{2+}-EGTA-ATPase of NAM extracted from mince washed with water was found with increasing storage times up to 7 days (P<0.05). Higher Mg^{2+}-EGTA-ATPase activity of NAM of mince washed with 20 ppm NaOCl was observed, when compared with that of mince washed with water (P<0.05). The result suggested that NaOCl might cause the changes in troponin-tropomyosin complex. Benjakul *et al.* (1997) found that the increase in Mg^{2+}-EGTA-ATPase was concomitant with the loss in Ca^{2+}-sensitivity, indicating the denatuation of tropomyosin C. Mg^{2+}-EGTA-ATPase is indicative of the integrity of troponin-tropomyosin complex (Benjakul *et al.*, 1997). From the result, washing mince with 20 ppm NaOCl possibly caused the partial denaturation of myosin as well as troponin-tropomyosin complex.

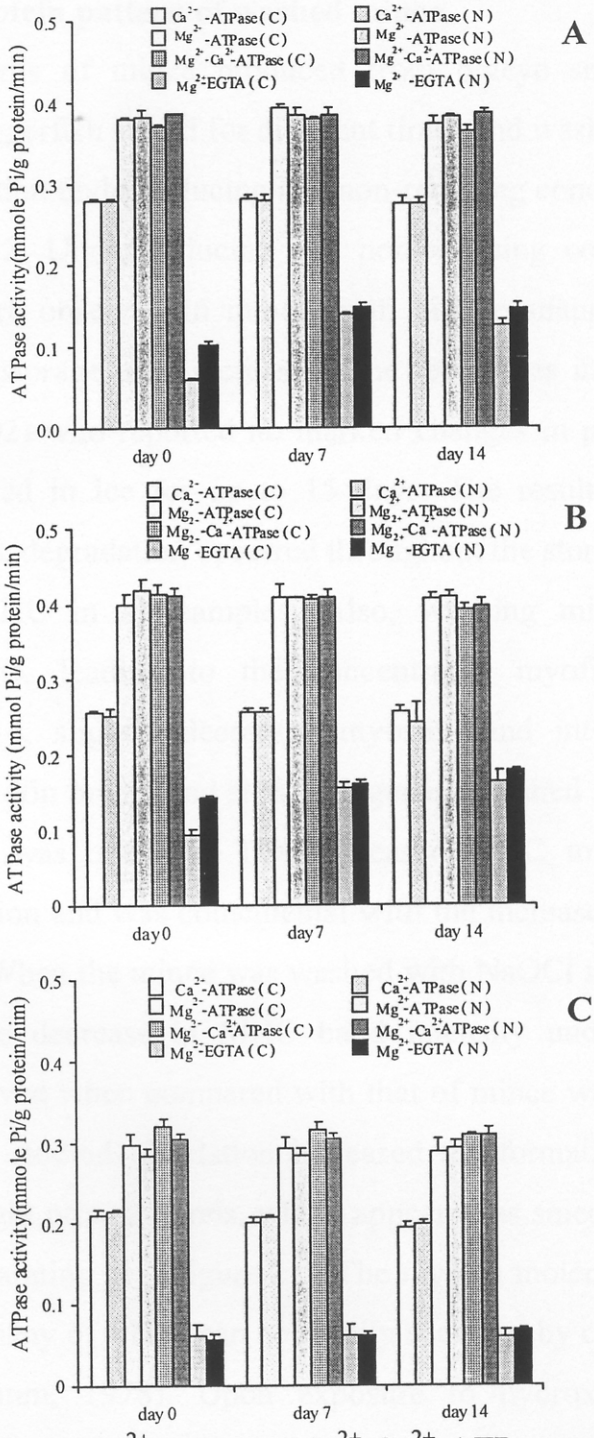

Figure 20 Ca^{2+}-ATPase, Mg^{2+}-ATPase, Mg^{2+}-Ca^{2+}-ATPase and Mg^{2+}-EGTA-ATPase activities of NAM extracted from bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with water (c) or 20 ppm NaOCl (N). Bars indicate the standard deviation from triplicate determinations.

1.10 Changes in protein pattern of washed mince

Protein patterns of mince produced from bigeye snapper, threadfin bream and starry triggerfish stored for different times and washed with water or 20 ppm NaOCl solution under reducing and non-reducing conditions are shown in Figure 21 and 22. Under reducing and non-reducing conditions, similar protein patterns were observed in mince from bigeye snapper washed with water when the ice storage time increased. The result was in agreement with Benjakul *et al.* (2002) who reported no marked changes in protein pattern of bigeye snapper stored in ice for up to 15 days. The results suggested that negligible proteolytic degradation occurred throughout the storage as evidenced by the retained MHC in all samples. Also, washing might remove the degradation products, leading to the concentrated myofibrillar proteins. However, at day 14, slightly decreased myosin band intensity of mince produced from threadfin bream and starry triggerfish washed with either water or NaOCl solution was obtained. The decreased MHC might result from proteolytic degradation and was coincidental with the increase in TCA-soluble peptide (Figure 8). When the mince was washed with NaOCl solution, which is oxidizing agent, the decrease in MHC band intensity under non-reducing condition was observed 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 (Figure 21). The myosin molecule has about 42 sulfhydryl groups, many of which can be readily accessed by chemical reagents (Hofmann and Hamm, 1978). Upon exposure to hydroxyl radicals, the sulfhydryl groups of MHC would be oxidized to form inter-molecular disulfide bonds (Liu and Xiong, 2000). The oxidation of protein accounts for the observation of more MHC loss in the oxidized myosin without β-mercaptoethanol. The decrease in MHC of mince washed with NaOCl solution was somehow coincidental with the increase in disulfide bonds and the

decrease in sulfhydryl group content (Figure 19). However, under reducing condition, no differences in MHC intensity were found between mince washed with both washing media. This result indicated that oxidizing agent might cause the oxidation 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 appropriate type and concentration of oxidizing agent in wash water might be associated with the increase in gel-forming ability.

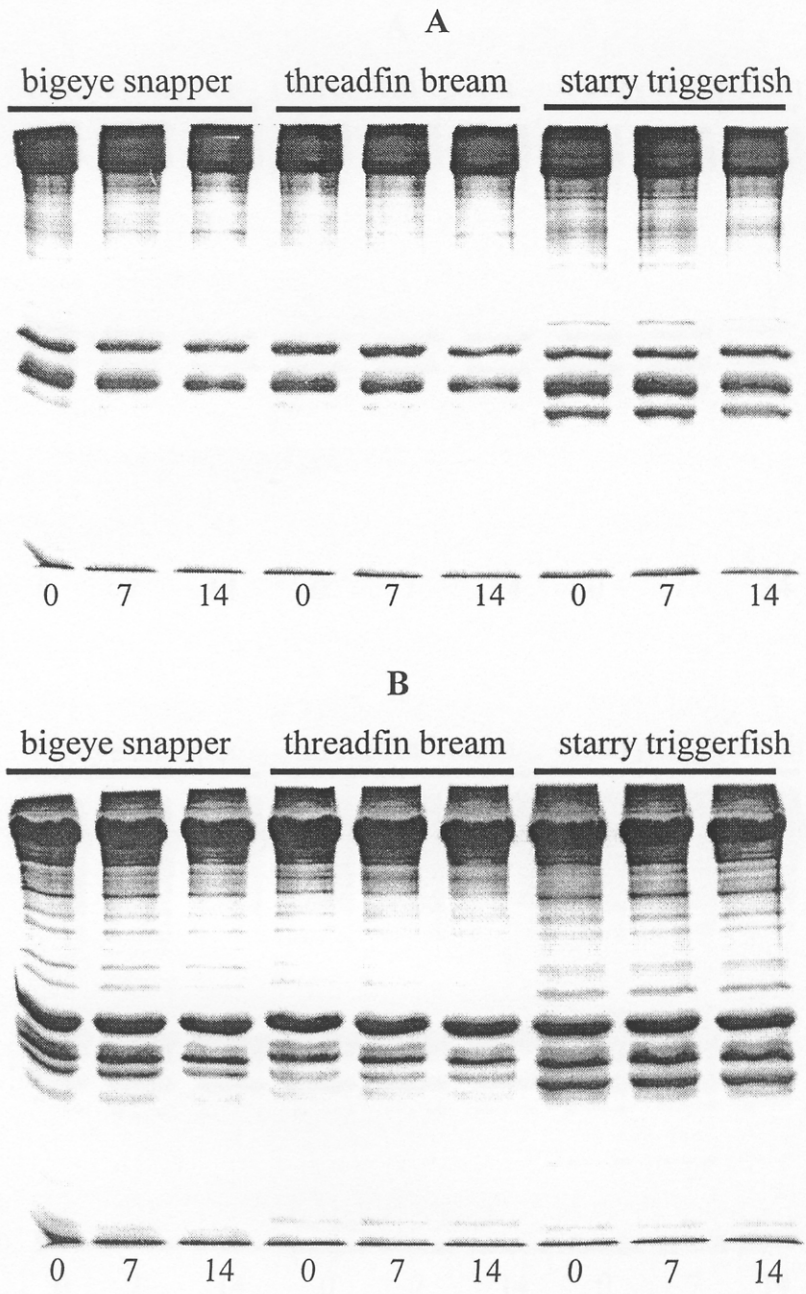

Figure 21 SDS-PAGE pattern of bigeye snapper, threadfin bream and starry triggerfish stored in ice for different times and washed with water. Numbers designate the storage time (days).

A; non-reducing, B; reducing

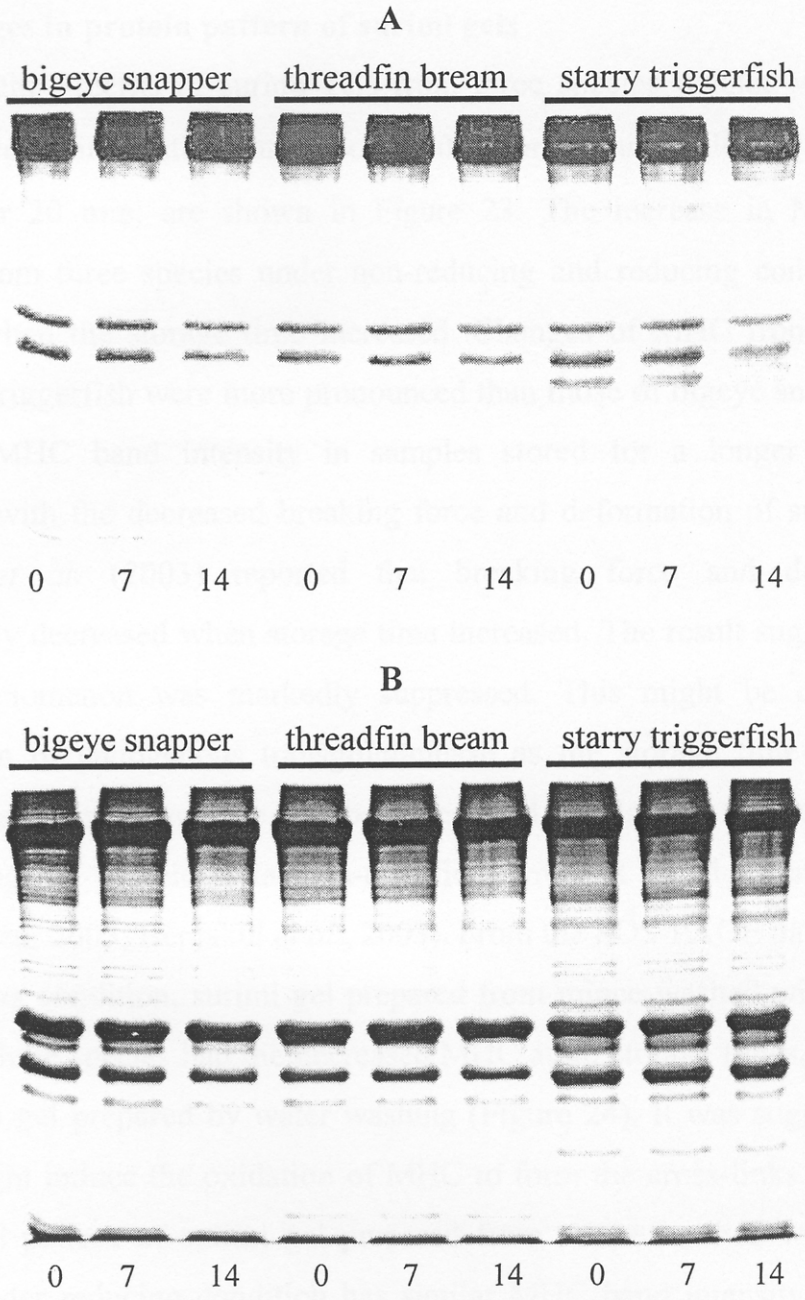

Figure 22 SDS-PAGE pattern of bigeye snapper, threadfin bream and starry triggerfish stored in ice for different times and washed with 20 ppm NaOCl. Numbers designate the storage time (days). A; non-reducing, B; reducing

1.11 Changes in protein pattern of surimi gels

Protein patterns of surimi gels from three species washed with water, and prepared by incubating surimi sol at 40°C for 30 min, followed by heating at 90°C for 20 min, are shown in Figure 23. The increase in MHC band intensity from three species under non-reducing and reducing condition was observed when the storage time increased. Changes of MHC from threadfin and starry triggerfish were more pronounced than those of bigeye snapper. The increased MHC band intensity in samples stored for a longer time was associated with the decreased breaking force and deformation of surimi gels. Benjakul *et al.* (2003) reported that breaking force and deformation continuously decreased when storage time increased. The result suggested that setting phenomenon was markedly suppressed. This might be due to the denaturation of endogenous transglutaminase as the storage time increased. Endogenous transglutaminase has been reported to play an essential role in cross-linking of proteins via non-disulfide covalent bond (Benjakul and Visessanguen, 2003; Benjakul *et al.*, 2003). From the SDS-PAGE pattern under non-reducing condition, surimi gel prepared from mince washed with 20 ppm NaOCl of three species had the decreased MHC at all storage times, compared with surimi gel prepared by water washing (Figure 24). It was suggested that NaOCl might induce the oxidation of MHC to form the cross-links. However, SDS-PAGE pattern of surimi gel prepared from mince washed with NaOCl solution under reducing condition has similar MHC band intensity compared from surimi gel water washed mince. The result indicated that washing with oxidizing agent did not show any influence on the setting of surimi. However, it caused the cross-linking of MHC via disulfide bond prior to gelation process. This might lead to the differences in gel property between surimi prepared by water and NaOCl washing.

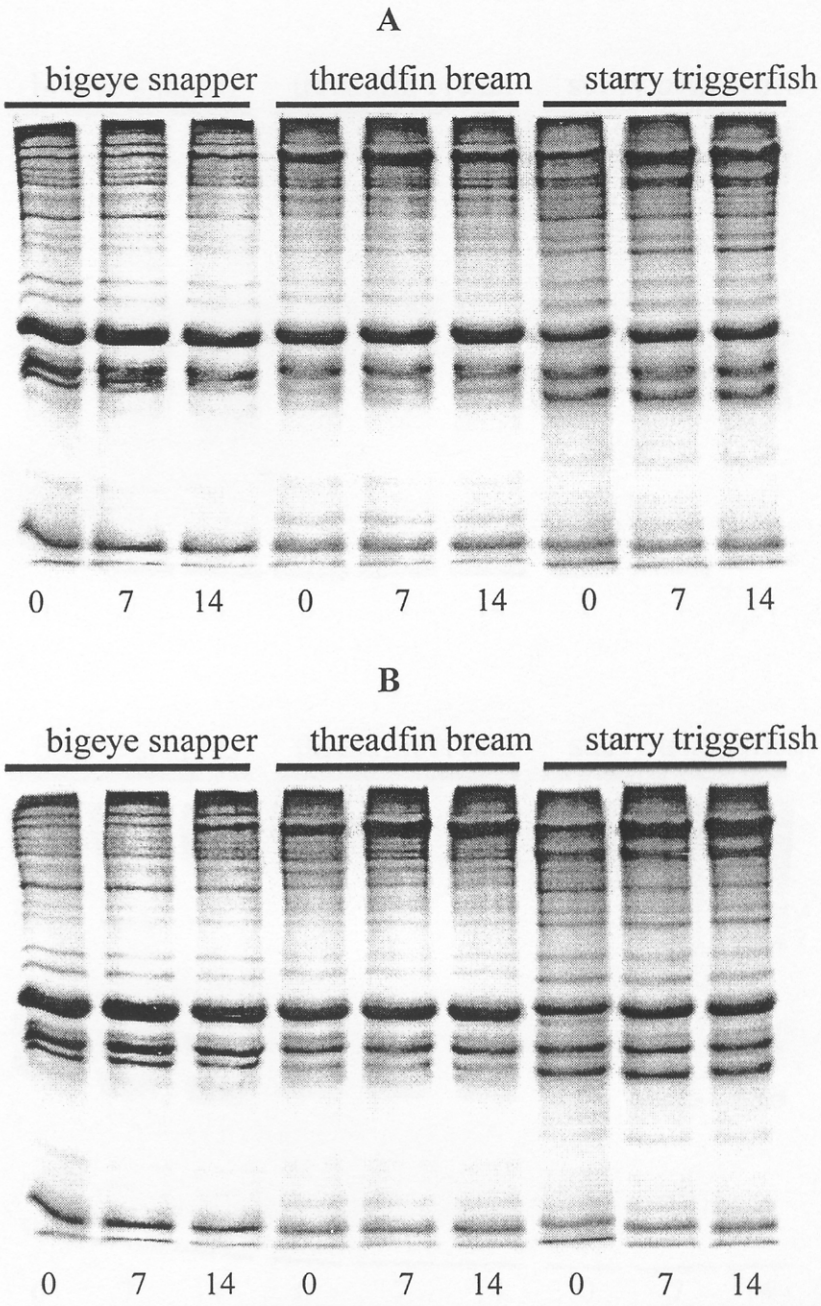

Figure 23 SDS-PAGE pattern of surimi gel of bigeye snapper, threadfin bream and starry triggerfish stored in ice for different times and washed with water. Numbers designate the storage time (days).

A; non-reducing, B; reducing

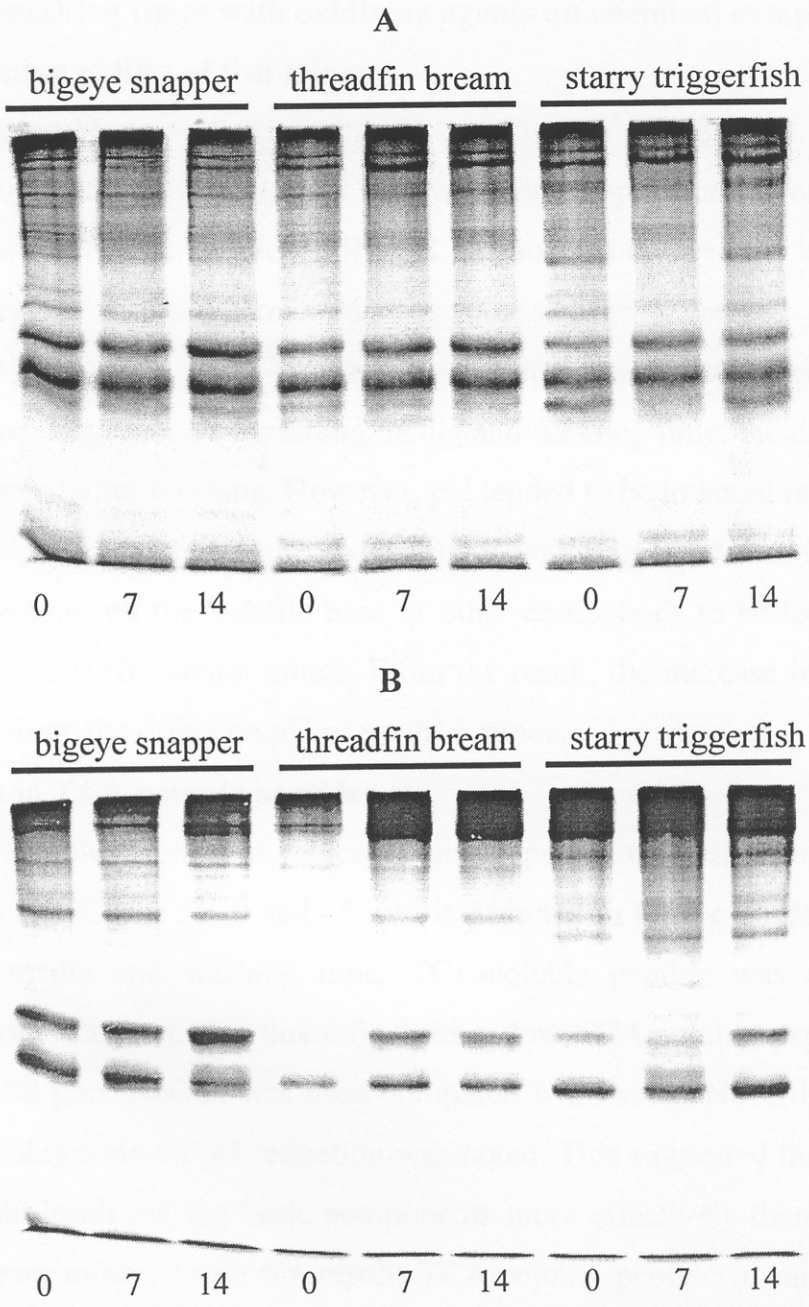

Figure 24 SDS-PAGE pattern of surimi gel of bigeye snapper, threadfin bream and starry triggerfish stored in ice for different times and washed with 20 ppm NaOCl. Numbers designate the storage time (days). A; non-reducing, B; reducing

2. Effect of washing times with oxidizing agents on chemical composition and gel-forming ability of fish mince

2.1 Changes in pH

The pH of both mince washed with water or 20 ppm NaOCl for different times is shown in Figure 25. Generally, pH of mince from threadfin bream and starry triggerfish increased markedly as the storage increased ($P<0.05$). However, no changes in pH of bigeye snapper mince were found when storage time increased. Regardless of washing media and washing time, the decrease in pH was observed after washing. However, pH tended to be lowered in threadfin bream mince when washing time increased. It was suggested that increasing washing time allowed the volatile base or other compounds to be leached out effectively in threadfin bream mince. From the result, the increase in washing time did not show the effect on pH of washed mince.

2.2 Changes in TCA-soluble peptides

TCA-soluble peptide of mince of three species washed with water or with 20 ppm NaOCl for 5, 10 and 15 min is depicted in Figure 26. Regardless of washing media and washing time, TCA-soluble peptide was decreased drastically with washing. For threadfin bream, low TCA-soluble peptide was found when 20 ppm NaOCl was used compared with water. Nevertheless, no effect of washing time on pH reduction was noted. This suggested that NaOCl solution might leach out the basic components more effectively than water in threadfin bream mince. From the result, TCA-soluble peptide in mince from three species increased sharply as the storage time increased. Bigeye snapper mince had the lower TCA-soluble peptide than mince from threadfin bream and starry triggerfish. Different autolysis was postulated among three species. However, proteolysis proceeded as the storage time increased. Benjakul *et al.* (1997) reported that the protein degradation of Pacific whiting was more intense as the storage time increased. Therefore, the increase in washing time had no effect on TCA-soluble peptide in mince.

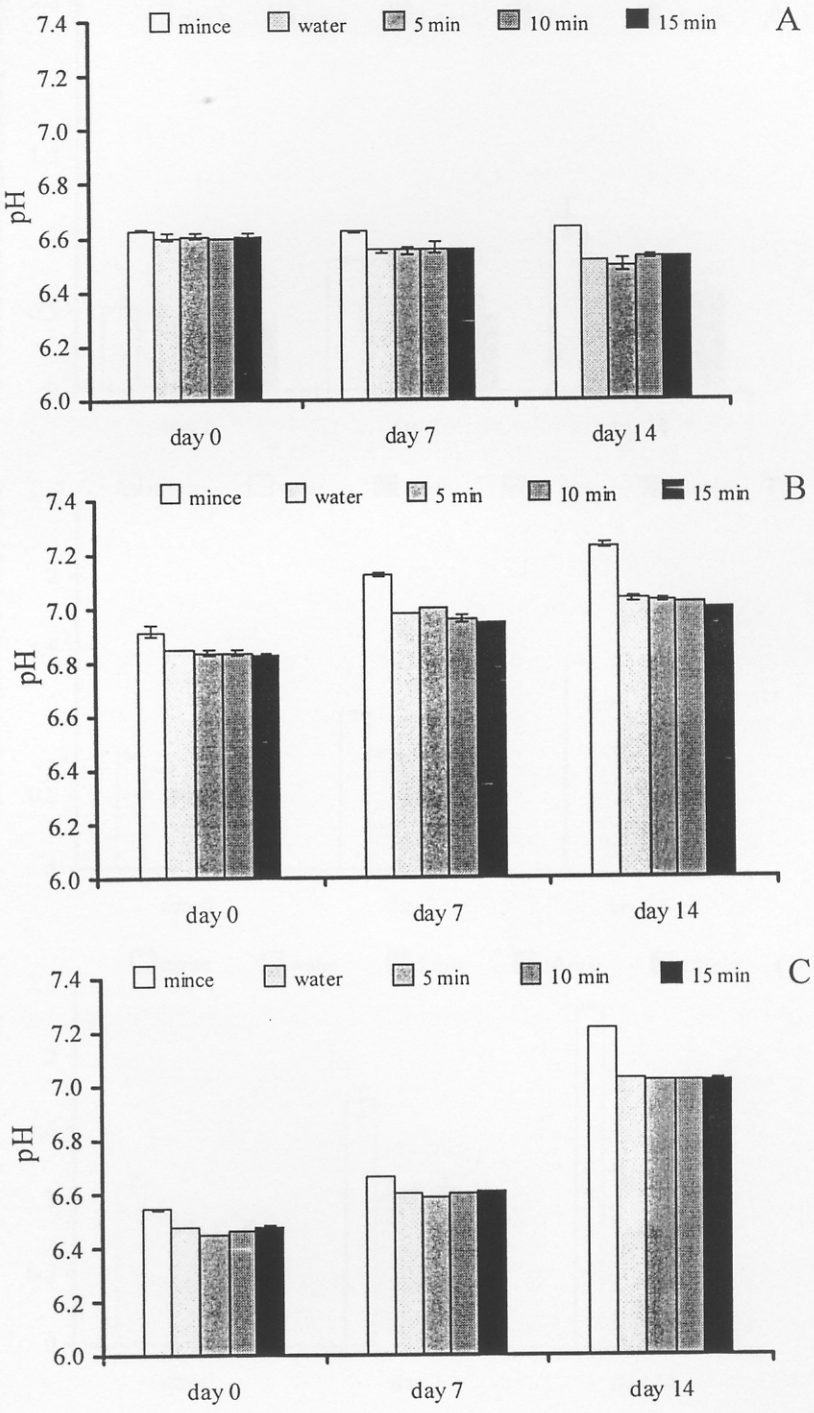

Figure 25 Changes in pH of bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with 20 ppm NaOCl for 5, 10 and 15 min. Bars indicate the standard deviation from triplicate determinations.

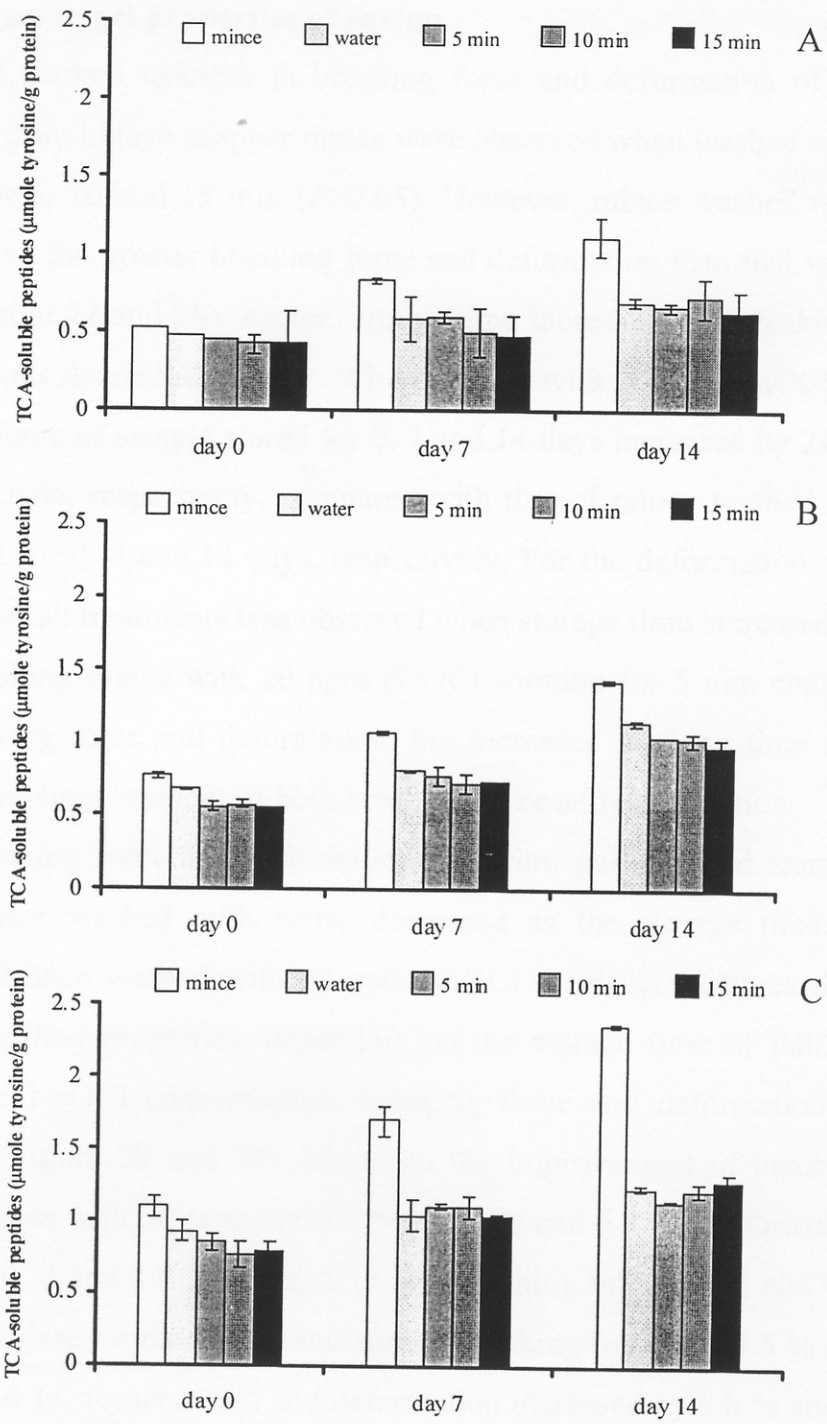

Figure 26 Changes in TCA-soluble peptides in bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with 20 ppm NaOCl for 5, 10 and 15 min. Bars indicate the standard deviation from triplicate determinations.

2.3 Changes in gel properties of surimi

No marked changes in breaking force and deformation of surimi gel produced from bigeye snapper mince were observed when washed with 20 ppm NaOCl for 5, 10 and 15 min (P>0.05). However, mince washed with NaOCl solution had the greater breaking force and deformation than that washed with water (Figure 27 and 28). As the storage time increased, the breaking force of all treatments decreased slightly. When washed with 20 ppm NaOCl for 5 min, breaking force of sample stored for 0, 7 and 14 days increased by 24.8 %, 13.4 % and 14.6 %, respectively, compared with that of mince washed with water and stored for 0, 7 and 14 days, respectively. For the deformation, only slight decrease for all treatments was observed when storage time increased. From the result, washing mince with 20 ppm NaOCl solution for 5 min could improve both breaking force and deformation, but increased washing time showed no effect on the improvement of both breaking force and deformation.

Breaking force and deformation of surimi gel prepared from threadfin bream mince washed with water decreased as the storage time increased (P<0.05). Mince washed with 20 ppm NaOCl for different times showed the different gelling properties, depending on the storage time of fish. With the appropriate NaOCl concentration, breaking force and deformation could be increased (Figure 29 and 30). Based on the improvement of breaking force, washing times with 20 ppm NaOCl were 5, 10 and 5 min for threadfin bream stored for 0, 7 and 14 days, respectively. Washing mince with NaOCl for the appropriate time resulted in the increase in breaking force by 21.5 % and 9.4 % at day 0 and 14, respectively, and deformation increased by 9.0 % and 1.0 % at day 0 and 14, respectively. Washing mince with NaOCl solution with different times might cause the changes in protein conformation or the cross-linking of proteins differently, depending on the freshness of fish. This led to the differences in gel improvement among fish stored for various times.

Breaking force and deformation of surimi gel produced from starry triggerfish mince washed with water decreased when storage time increased. The highest breaking force and deformation were found when the mince was washed with 20 ppm NaOCl for 5, 15 and 5 min for starry triggerfish stored for 0, 7 and 14 days, respectively (Figure 31 and 32). The increasing washing time had no effect on both breaking force and deformation of mince produced from fish stored for 0 and 14 fays. From the result, it can be inferred that washing with 20 ppm NaOCl resulted in gel improvement differently, depending on washing time.

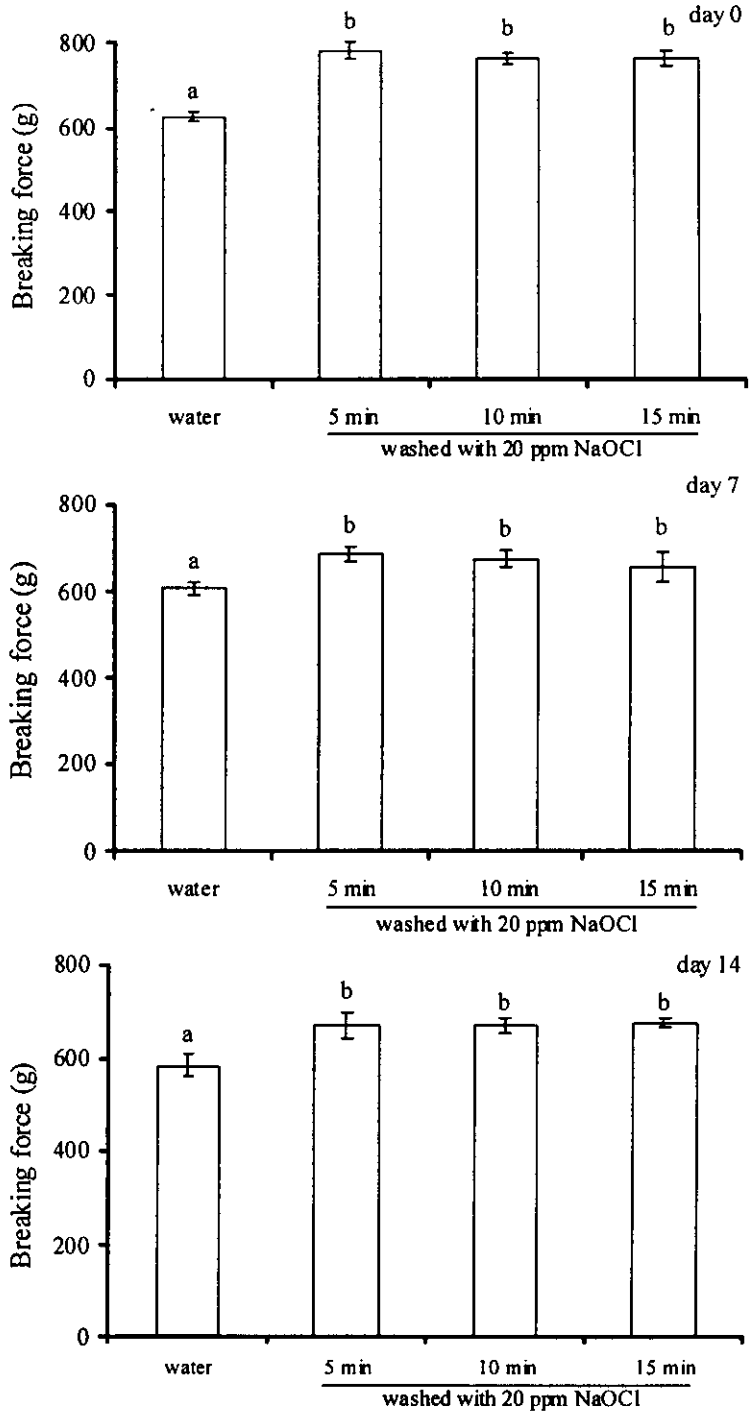

Figure 27 Changes in breaking force of gel of surimi produced from bigeye snapper stored in ice for different times and washed with water or 20 ppm NaOCl for different times. Bars indicate the standard deviation from five determinations.

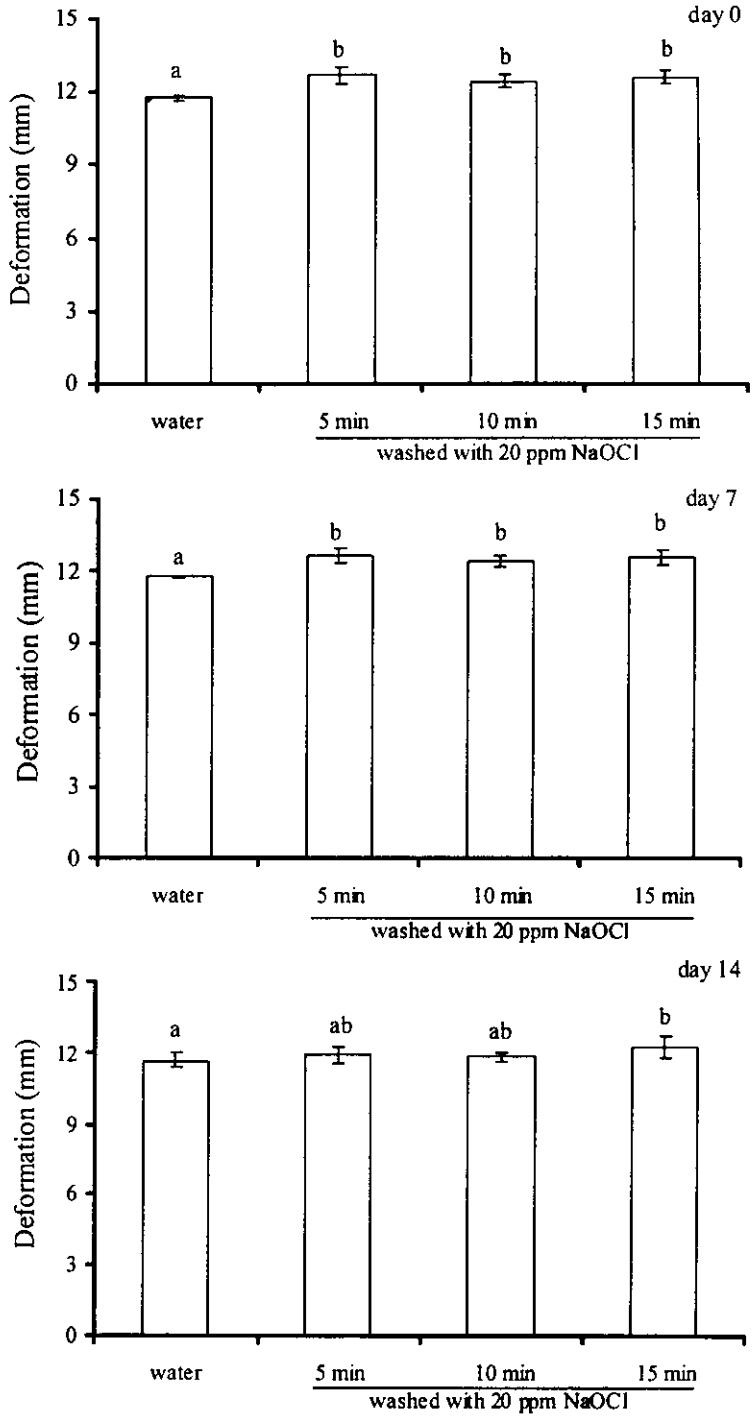

Figure 28 Changes in deformation of gel of surimi produced from bigeye snapper stored in ice for different times and washed with water or 20 ppm NaOCl for different times. Bars indicate the standard deviation from five determinations.

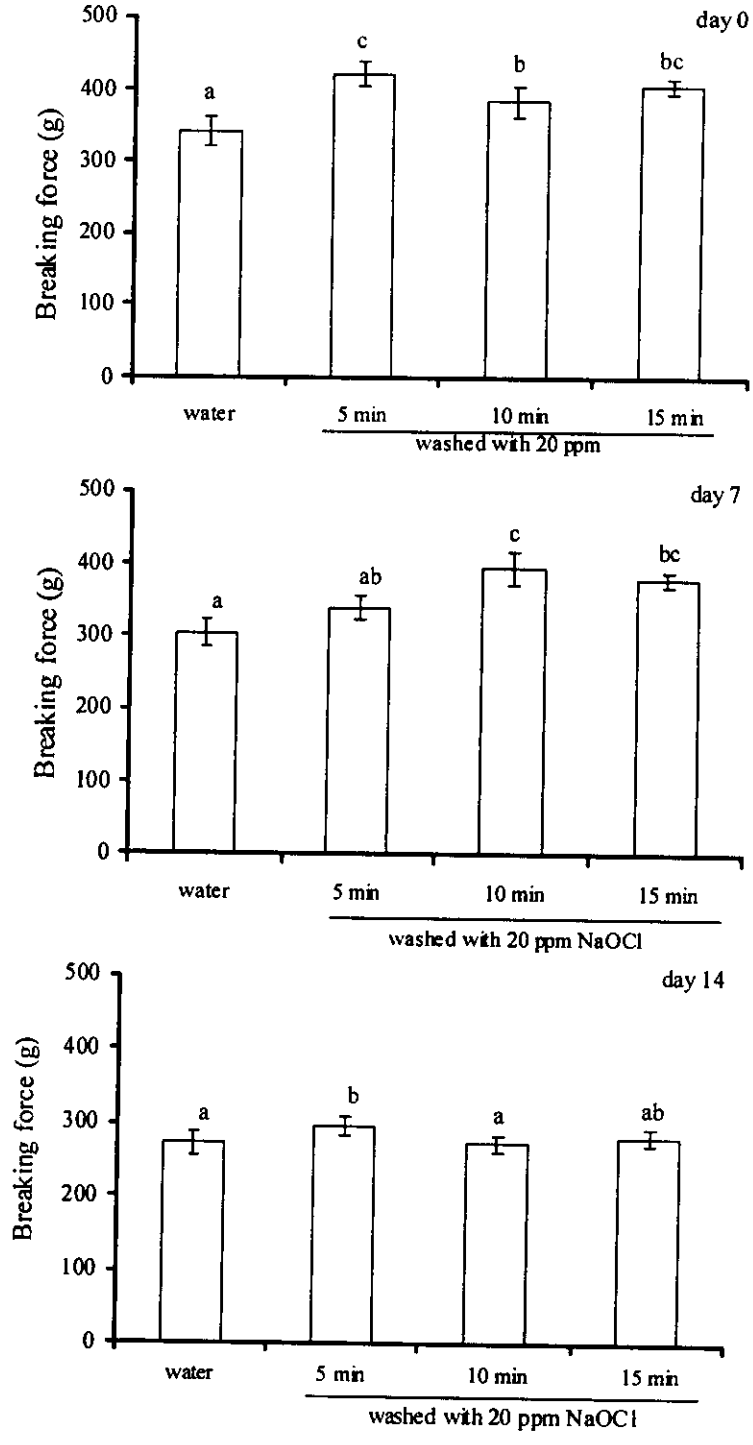

Figure 29 Changes in breaking force of gel of surimi produced from threadfin bream stored in ice for different times and washed with water or 20 ppm NaOCl for different times. Bars indicate the standard deviation from five determinations.

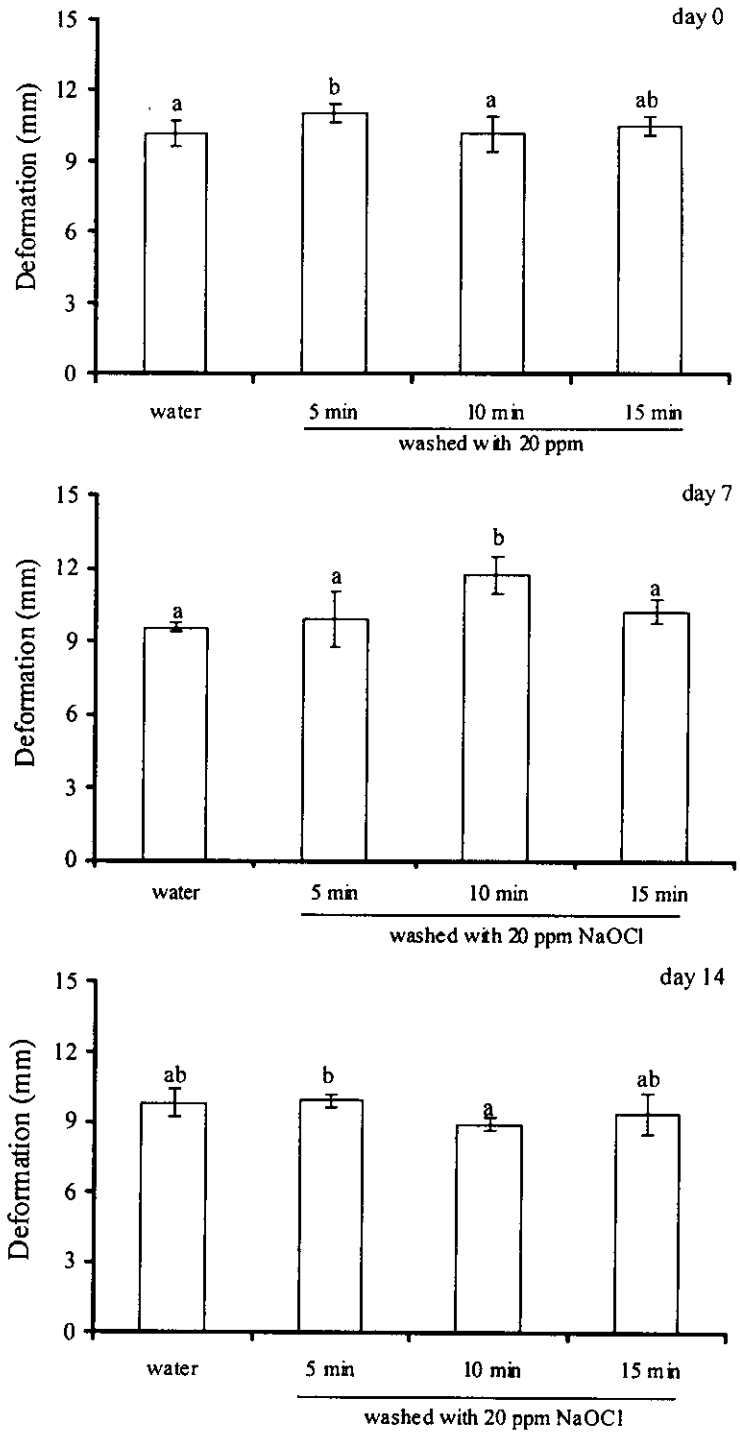

Figure 30 Changes in deformation of gel of surimi produced from threadfin bream stored in ice for different times and washed with water or 20 ppm NaOCl for different times. Bars indicate the standard deviation from five determinations.

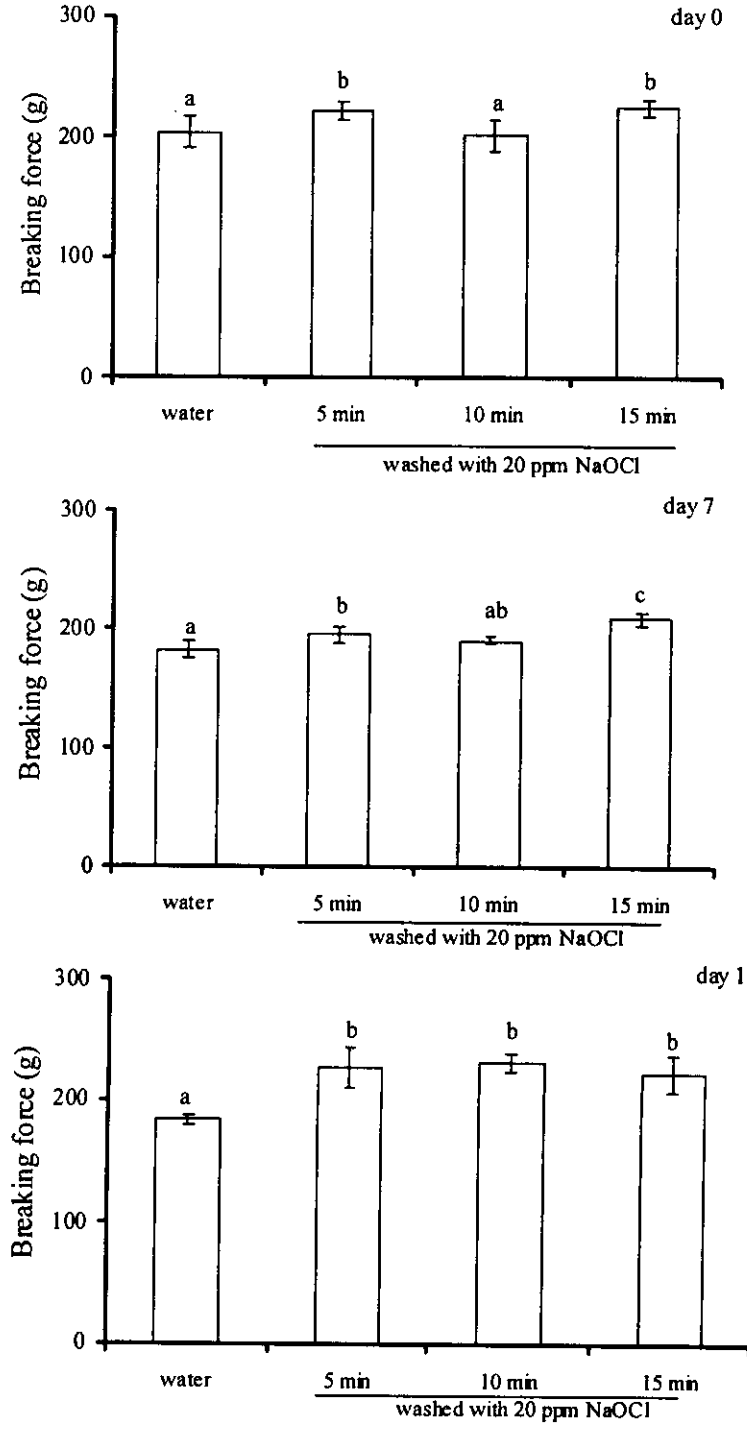

Figure 31 Changes in breaking force of gel of surimi produced from starry triggerfish stored in ice for different times and washed with water or 20 ppm NaOCl for different times. Bars indicate the standard deviation from five determinations.

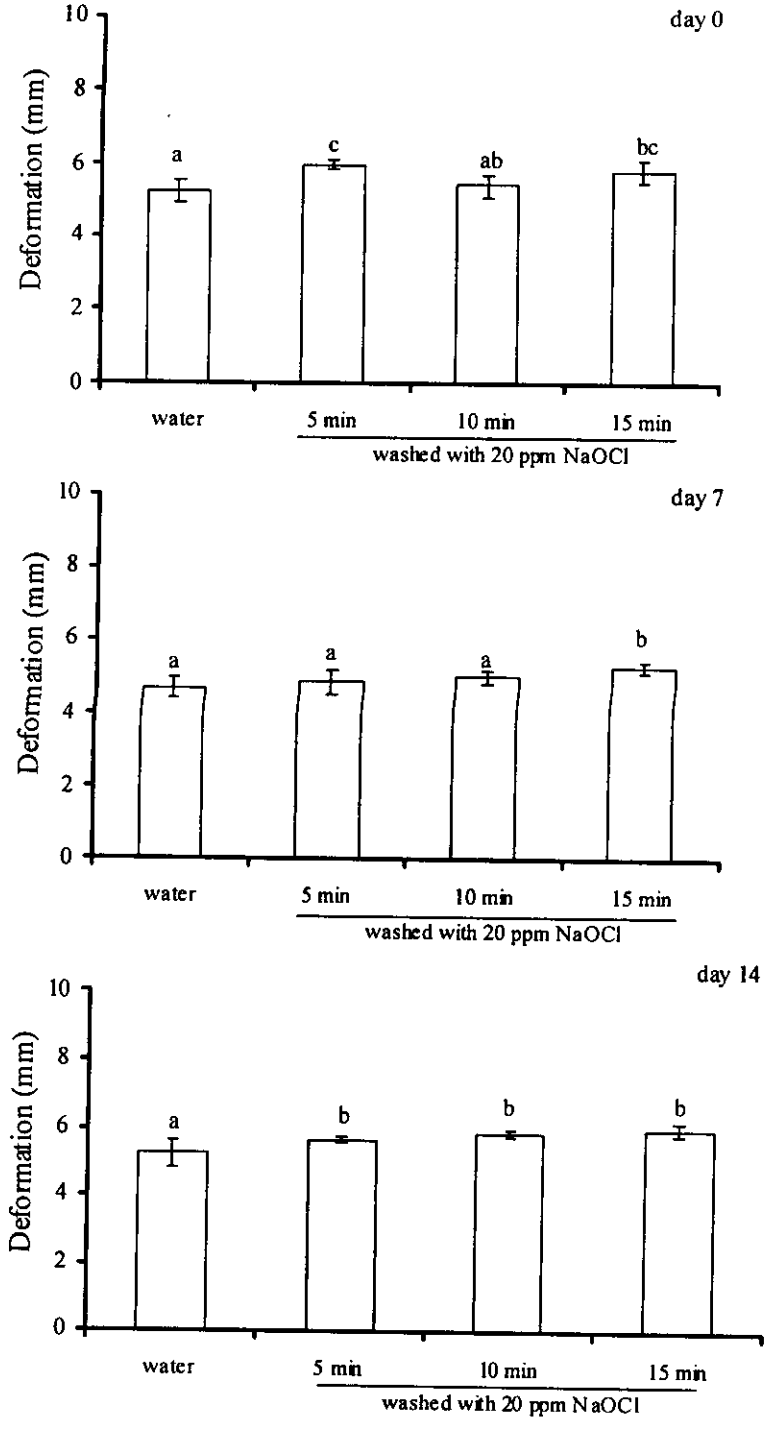

Figure 32 Changes in deformation of gel of surimi produced from starry triggerfish stored in ice for different times and washed with water or 20 ppm NaOCl for different times. Bars indicate the standard deviation from five determinations.

2.4 Changes in whiteness

Surimi gel prepared from bigeye snapper mince washed with water had the decreased whiteness when the storage time increased. No marked differences in whiteness were observed between gels from mince washed with water and 20 ppm NaOCl for different washing times (Table 4). For the threadfin bream, surimi gel had the slight decrease in whiteness when the storage time increased. NaOCl showed no obvious effect on whiteness of gel from this species (Table 5). The whiteness of starry triggerfish decreased sharply within the first 7 days of storage. Washing with NaOCl for different times had no marked influence on the whiteness of gels (Table 6). From the result, using NaOCl as washing medium showed negligible effect on whiteness of gel from all three species.

Table 4 Whiteness of surimi gels produced from bigeye snapper during iced storage and washed with 20 ppm NaOCl for different times.

	storage time		
washing time	day 0	day 7	day 14
water	76.33±0.17 c	75.15±0.57 a	73.58±0.14 b
5 min	75.29±0.09 a	75.23±0.20 a	72.57±0.40 a
10 min	76.59±0.06 c	74.87±0.17 a	73.51±0.23 b
15 min	75.99±0.20 b	75.09±0.34 a	73.61±0.30 b

*Values are mean ± standard deviation (n=4). Values with the same letter in the same column are not significantly different ($P>0.05$).

Table 5 Whiteness of surimi gels produced from threadfin bream during iced storage and washed with 20 ppm NaOCl for different times.

	storage time		
washing time	day 0	day 7	day 14
water	77.76±0.20 a	78.03±0.33 a	76.87±0.14 a
5 min	77.59±0.14 a	77.93±0.21 a	78.13±0.20 b
10 min	77.59±0.35 a	78.01±0.08 a	77.32±0.18 c
15 min	78.73±0.23 b	78.15±0.26 a	77.02±0.08 ab

*Values are mean ± standard deviation (n=4). Values with the same letter in the same column are not significantly different ($P>0.05$).

Table 6 Whiteness of surimi gels produced from starry triggerfish during iced storage and washed with 20 ppm NaOCl for different times.

	storage time		
washing time	day 0	day 7	day 14
water	80.03±0.15 c	76.00±0.14 b	75.42±0.05 b
5 min	79.39±0.18 a	75.31±0.13 a	75.32±0.01 a
10 min	79.29±0.03 a	76.72±0.17 c	75.12±0.02 a
15 min	79.66±0.07 b	76.20±0.35 b	75.81±0.28 c

*Values are mean ± standard deviation (n=4). Values with the same letter in the same column are not significantly different ($P>0.05$).

2.5 Changes in TBARS

TBARS of bigeye snapper, threadfin bream and starry triggerfish mince and mince washed with water or 20 ppm NaOCl solution for different times are depicted in Figure 33. Constant TBARS of mince from threadfin bream and starry triggerfish was observed throughout the storage. The increased TBARS content was found in bigeye snapper stored in ice for 14 days. Generally, mince washed with water or NaOCl solution had the increased TBARS, compared with unwashed mince. During washing process, lipid in mince might be exposed to oxygen and underwent oxidation easily. Lipid and membrane lipids

in fish have been reported to contain the high content of polyunsaturated fatty acids such as EPA and DHA, which are prone to oxidation (Leaf and Weber, 1988). Mince washed with oxidizing agent exhibited the greater TBARS than mince washed water, suggesting the role of NaOCl in acceleration of lipid oxidation. Hwang and Regenstein (1995) found that the TBARS of Atlantic mackerel mince increased during the storage at 0°C for 26 days. Sista *et al.* (2000) reported that in iron ascorbate systems, large increases on hydroperoxides and TBARS occurred during the initial stage of incubation. From the result, the increased washing time had no effect on TBARS of mince. However, the washing with NaOCl solution resulted in increased oxidation of lipid.

2.6 Changes in TVB and TMA contents

TVB and TMA contents of mince and washed mince increased when the storage times increased for all three species (Figure 34 and 35). The TVB contents of mince (unwashed) from bigeye snapper, threadfin bream and starry triggerfish were 6.67, 6.36 and 11.96 (mg N/100g) at day 0, and at day 14, TVB contents were 8.52, 17.65 and 45.70 (mg N/100g), respectively. However, no substantial differences in TVB content were found between mince washed with water and NaOCl solution for 5 min. For TMA content, similar result was observed, when compared with TVB results. Regardless of washing media, TMA content was markedly reduced. From the result, the washing with water or 20 ppm NaOCl for 5 min could reduce TVB and TMA contents in mince from all species studied. TBV and TMA cause the offensive odor to the mince obtained. TMA was associated with the fishy odor (Gram and Huss, 1996; Huss, 1995). Thus, the removal of those compounds by washing either with water or 20 ppm NaOCl could reduce the undesirable odor from the mince.

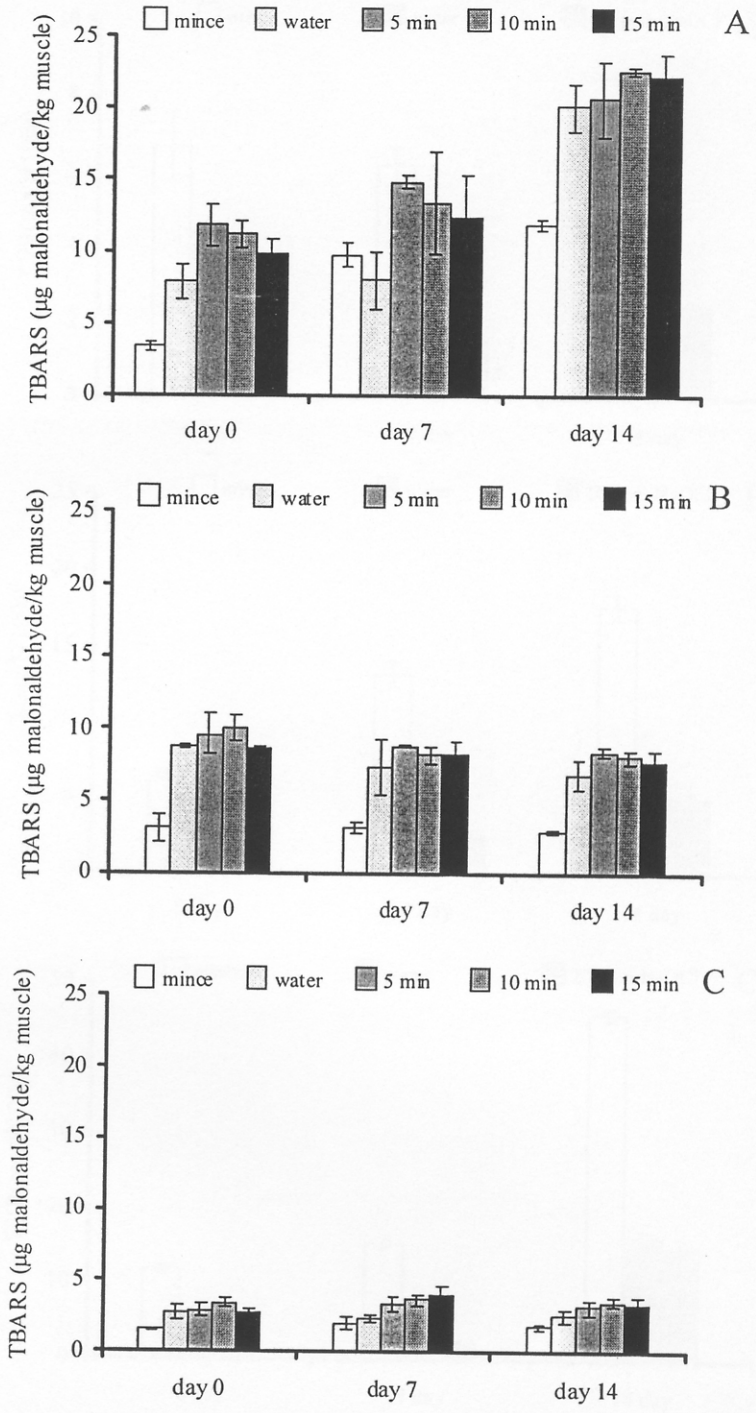

Figure 33 Changes of TBARS in bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with 20 ppm NaOCl for 5, 10 and 15 min. Bars indicate the standard deviation from triplicate determinations.

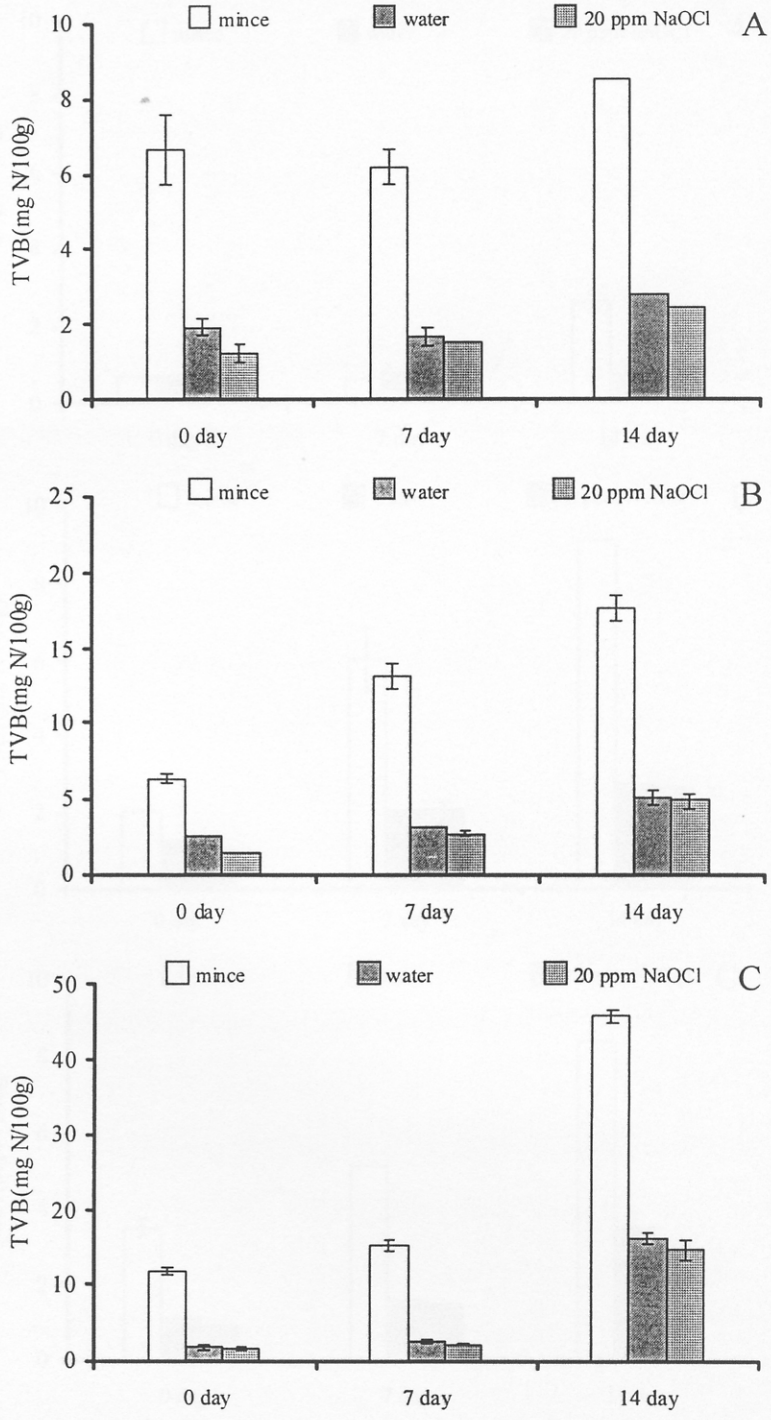

Figure 34 Changes of TVB in bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with 20 ppm NaOCl for 5 min. Bars indicate the standard deviation from triplicate determinations.

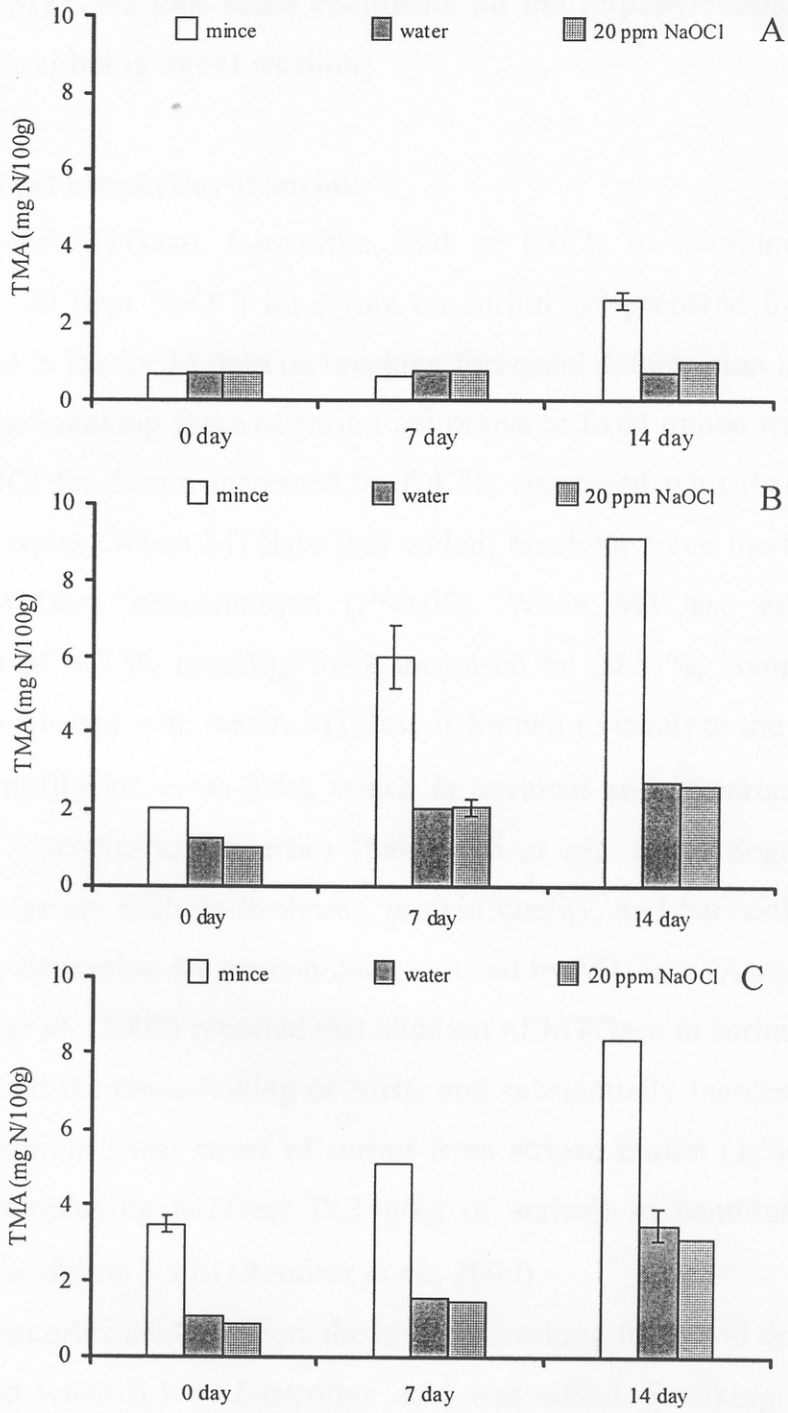

Figure 35 Changes of TMA in bigeye snapper (A), threadfin bream (B) and starry triggerfish (C) mince stored in ice for different times and washed with 20 ppm NaOCl for 5 min. Bars indicate the standard deviation from triplicate determinations.

3. Effect of MTGase and some chemicals on gel improvement of surimi produced by oxidizing agent washing

3.1 Effect on gel properties of surimi

Effect of MTGase, *L*-ascorbic acid or $CaCl_2$ in combination with washing with 20 ppm NaOCl for 5 min on surimi gel prepared from bigeye snapper stored in ice for 14 days on breaking force and deformation is shown in Figure 36. The breaking force of surimi gel prepared from mince washed with 20 ppm NaOCl for 5 min increased by 8.4 %, compared with that of mince washed with water. When MTGase was added, breaking force increased with increasing MTGase concentration ($P<0.05$). When MTGase added at a concentration of 0.3 %, breaking force increased by 24.3 %, compared with that of mince washed with water. MTGase is known to catalyze the formation of ε-(γ-glutamyl)lysine cross-links, which is covalent and important for gel-forming and viscoelastic properties (Sakamoto *et al.*, 1995; Seguro *et al.*, 1995). Some factors such as freshness, protein quality, and harvesting season were found to determine the cross-linking induced by MTGase (Asagami *et al.*, 1995). Hsieh *et al.* (2002) reported that addition of MTGase in surimi gel from mackerel caused the cross-linking of MHC and substantially increased the gel strength. Maximum shear stress of surimi from striped mullet (156 kPa) was obtained by employing MTGase (9.3 g/kg of surimi) in combination with setting (37°C and time 3.9 h) (Ramirez *et al.*, 2000).

For *L*-ascorbic acid addition, the highest breaking force and deformation were observed when 0.1 % *L*-ascorbic acid was added. Breaking force and deformation increased by 22.1 % and 10.3 %, respectively, compared with those of mince washed with water. However, the decrease in breaking force and deformation was found with increasing *L*-ascorbic acid (0.2 or 0.3 %). This might be due to the excessive cross-linking induced by *L*-ascorbic acid. *L*-ascorbic acid is commonly used in bread dough to improve textural properties

by the formation disulfide bonds (-S-S-) through the oxidation of sulfhydryl (-SH) groups (Park, 2000). Lee *et al.* (1992) found that sodium-*L*-ascorbate significantly improved the compressive force of the gel and the sensory firmness of molded and fiberized products with the maximal effect at a level of 0.2 %.

Addition of $CaCl_2$ up to 50 mmole/kg increased both breaking force and deformation ($P<0.05$). When the concentration of 100 mmole was used, slight decrease in breaking force was observed. The result suggested that calcium ion might activate endogenous TGase, leading to the greater cross-linking via non-disulfide covalent bond. Fish TGase has differing sensitivities to calcium ion (Ashie and Lanier, 2000). Walleye pollack TGase required 3 mM calcium ion, while carp muscle TGase required 5 mM calcium ion for full activation (Kishi *et al.*, 1991). Therefore, effectiveness of calcium chloride in enhancing setting response depends on fish species. Benjakul *et al.* (2004) reported that addition of excessive amount of calcium chloride, especially 120 mmole/kg, resulted in the decreases in breaking force in all surimi (from bigeye snapper, threadfin bream, barracuda and bigeye croaker). Surimi gels from striped mullet had maximal shear stress (89.6 kPa) by addition of 0.4 % $CaCl_2$ (Ramirez *et al.*, 2003). At high levels of calcium chloride, calcium or chloride ion might cause changes in protein conformation. Ions interact with oppositely charged groups on protein molecules to form a double layer of ionic groups, leading to the decreases in electrostatic interactions between protein molecules (Vojdani, 1996). From the result, addition of MTGase, *L*-ascorbic acid or $CaCl_2$ levels of 0.2 %, 0.1 % and 50 mmole/kg increased the gel strength of surimi from bigeye snapper effectively.

For threadfin bream surimi, breaking force and deformation of surimi gel prepared from fish stored in ice for 14 days increased by 33.2 and 14.5 %, when 20 ppm NaOCl was used as a washing medium (Figure 37). Addition of MTGase increased breaking force and deformation in a concentration

dependent manner. MTGase at a level of 0.3 % (w/w) could increase breaking force and deformation by 145.8 % and 47.8 %, respectively, compared with the control. *L*-ascorbic acid at levels of 0.1 and 0.2 % increased breaking force and deformation of surimi gel. Addition of 0.3 % *L*-ascorbic acid caused the slight decrease in breaking force. The decrease in gel strength was found when high concentration of *L*-ascorbic acid was used. This might be due to the decrease in pH of surimi, leading to the denaturation of muscle protein. For $CaCl_2$ added surimi gel, $CaCl_2$ at all levels tested had no effect on both breaking force and deformation. This was possibly due to the low TGase activity. Additionally, those TGase might be present in the fully active form. As a consequence, no additional calcium ion was needed.

Breaking force and deformation of surimi prepared from starry triggerfish increased from 183.7 g and 5.4 mm (washed with water) to 227.3 g and 5.8 mm when 20 ppm NaOCl was used as the washing medium (Figure 38). When MTGase was added up to 0.2 %, breaking force and deformation increased. However, no increase in breaking force was found when MTGase at level of 0.3 % was added. From the result, the addition of MTGase could improve gel strength as the appropriate concentration was used. However, addition of *L*-ascorbic acid or $CaCl_2$ at all levels used had no effect on breaking force and deformation ($P>0.05$). Therefore, the MTGase at 0.2 % (w/w) effectively increased gel strength of surimi from starry triggerfish.

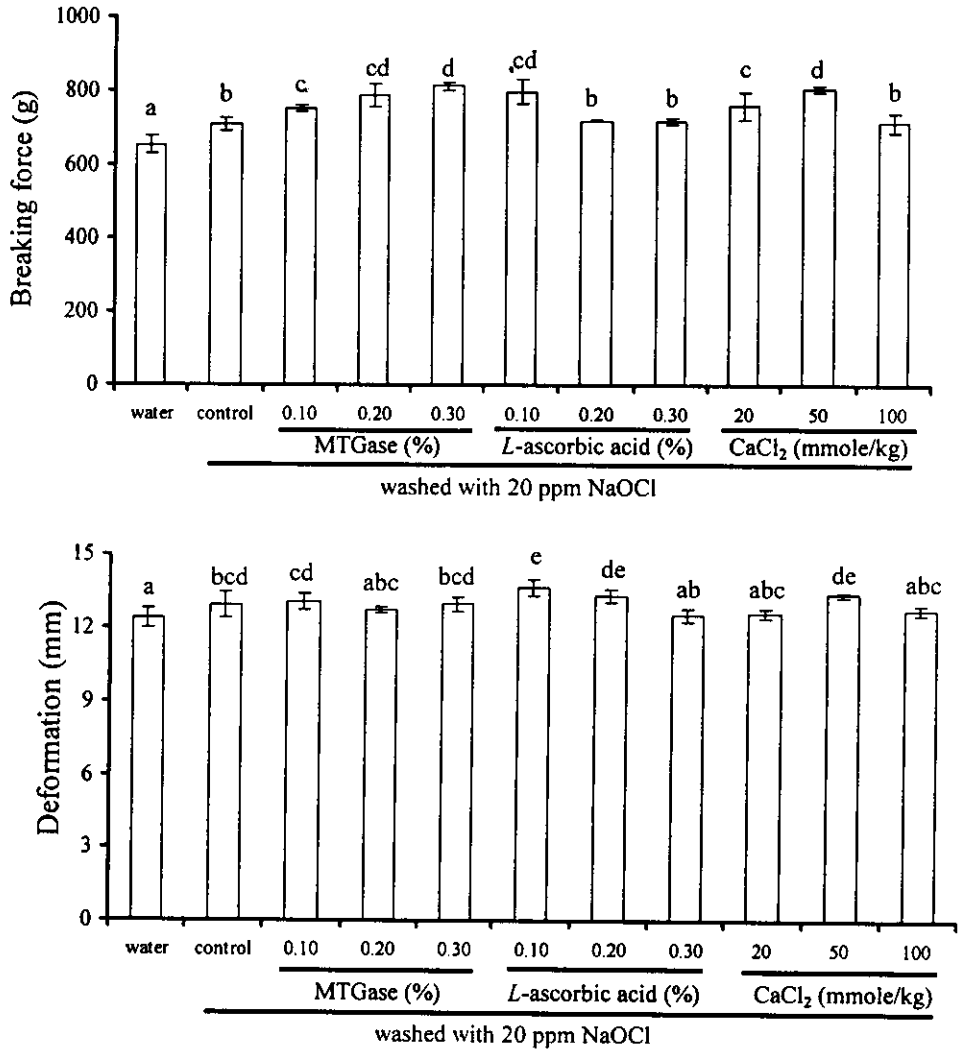

Figure 36 Changes in breaking force and deformation of the gel of bigeye snapper surimi prepared by 20 ppm NaOCl washing in combination with MTGase, *L*-ascorbic acid or $CaCl_2$ addition. Bigeye snapper stored in ice for 14 days was used for surimi preparation.
Bars represent the standard deviation from five determinations.

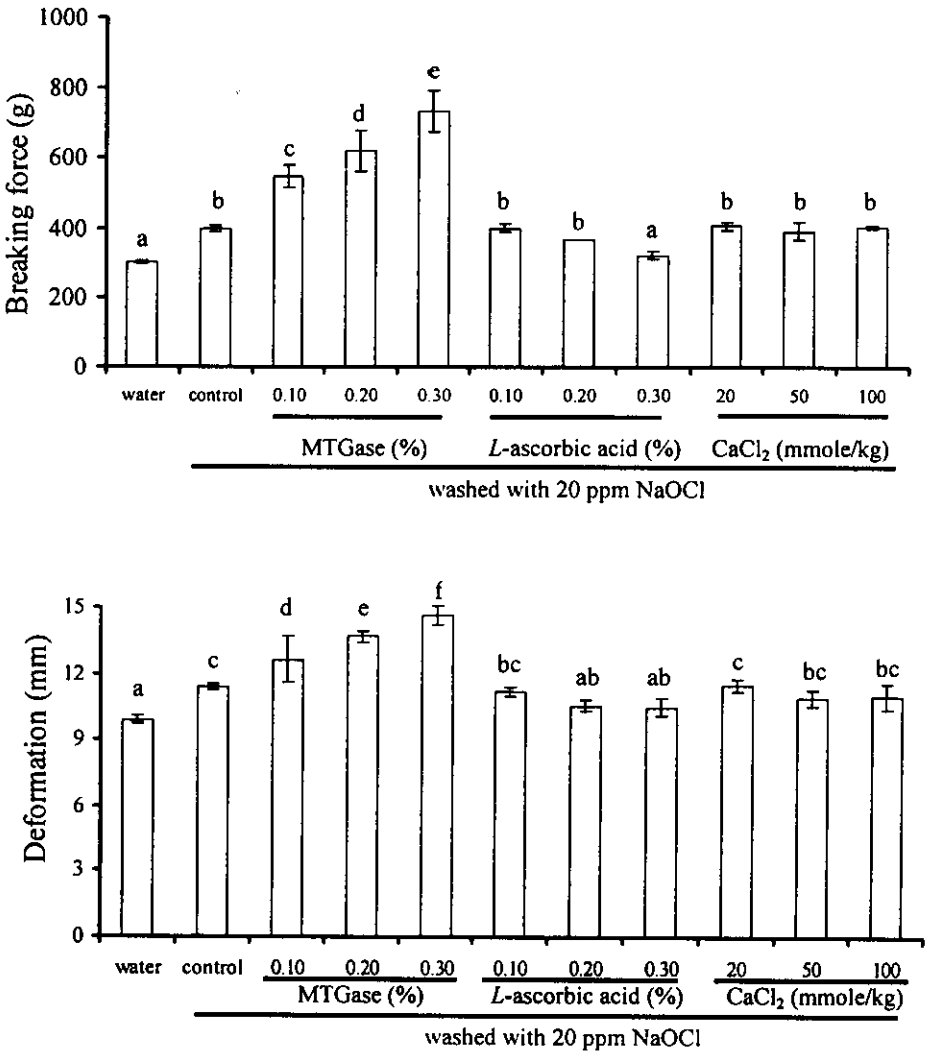

Figure 37 Changes in breaking force and deformation of the gel of threadfin bream surimi prepared by 20 ppm NaOCl washing in combination with MTGase, *L*-ascorbic acid or $CaCl_2$ addition. Threadfin bream stored in ice for 14 days was used for surimi preparation.

Bars represent the standard deviation from five determinations.

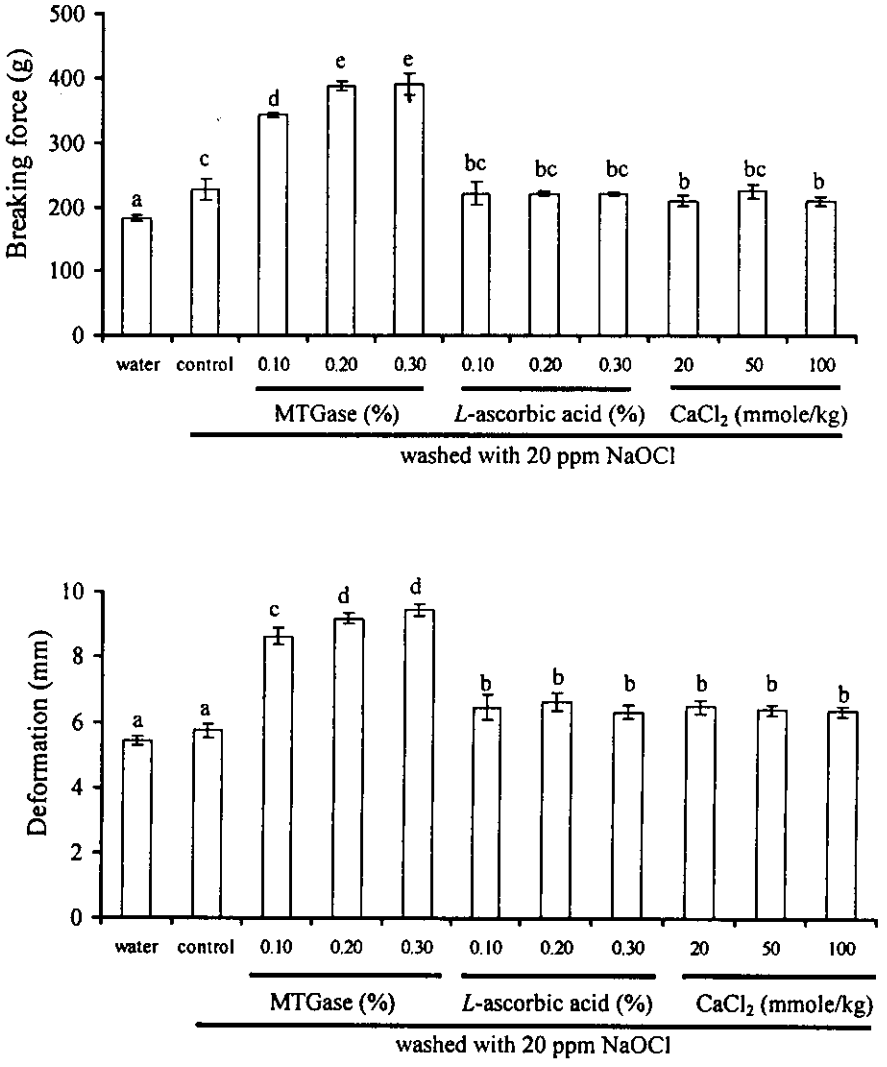

Figure 38 Changes in breaking force and deformation of the gel of starry triggerfish surimi prepared by 20 ppm NaOCl washing in combination with MTGase, *L*-ascorbic acid or $CaCl_2$ addition. Starry triggerfish stored in ice for 14 days was used for surimi preparation. Bars represent the standard deviation from five determinations.

3.2 Effect on protein patterns of surimi gel

Protein patterns of bigeye snapper surimi gel added with MTGase or $CaCl_2$ revealed that MHC band intensity decreased when the concentrations of both MTGase and $CaCl_2$ increased under both non-reducing and reducing conditions. No mark changes in protein pattern were observed with gel added with *L*-ascorbic acid. However, band intensity of MHC was increased when tested under reducing condition, suggesting that *L*-ascorbic acid might induce the cross-linking via disulfide bond. The decrease in MHC band intensity was generally coincidental with the increase in gel strength (Figure 39). MTGase catalyzes the formation of ε-(γ-glutamyl)lysine cross-links (Sakamoto *et al.*, 1995; Seguro *et al.*, 1995). $CaCl_2$ has been known to catalyze the formation of ε-(γ-glutamyl)lysine cross-links by endogenous TGase. *L*-ascorbic acid improves the textural properties by the formation of disulfide bonds (-S-S-) through the oxidation of sulfhydryl (-SH) groups (Park, 2000).

For protein patterns of surimi gel from threadfin bream and starry triggerfish added with MTGase, similar patterns were observed when compared with those of bigeye snapper surimi gel. When MTGase concentration increased, the marked decrease in MHC band intensity was noticeable (Figure 40 and 41). The decreased MHC band intensity was in agreement with the increase in gel strength. However, no changes in MHC band intensity was found in presence of $CaCl_2$. It indicated that $CaCl_2$ addition did not affect the formation of ε-(γ-glutamyl)lysine cross-links by endogenous TGase. The result was concomitant with breaking force, which was not changed with $CaCl_2$ addition. No changes in protein patterns were found with addition of *L*-ascorbic acid which was coincidental with the no changes in breaking force or lowered breaking force. *L*-ascorbic acid at higher concentration decreased the cross-linking of protein, since it could denature the protein by lowering the pH or oxidizing the cysteine in the active site of TGase. From the result, MTGase addition improved gel strength of all species most potentially, while *L*-ascorbic

acid and $CaCl_2$ improved gel strength of only bigeye snapper surimi. Therefore, the efficacy of MTGase and chemicals in gel improvement depended on fish species and quality.

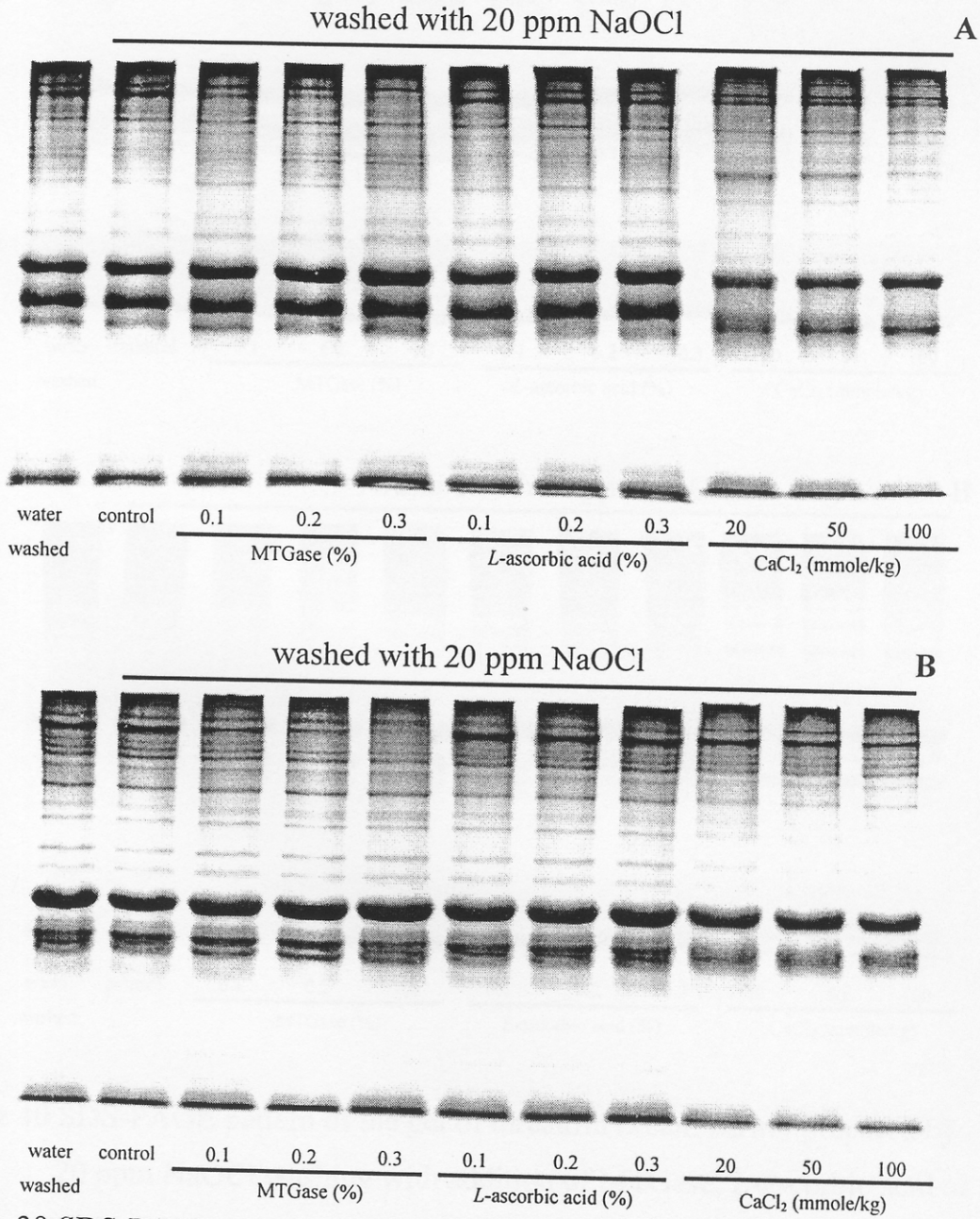

Figure 39 SDS-PAGE pattern of the gel of bigeye snapper surimi prepared by 20 ppm NaOCl washing with addition of MTGase, *L*-ascorbic acid or $CaCl_2$. Bigeye snapper stored in ice for 14 days was used for surimi preparation. A; non-reducing : B; reducing.

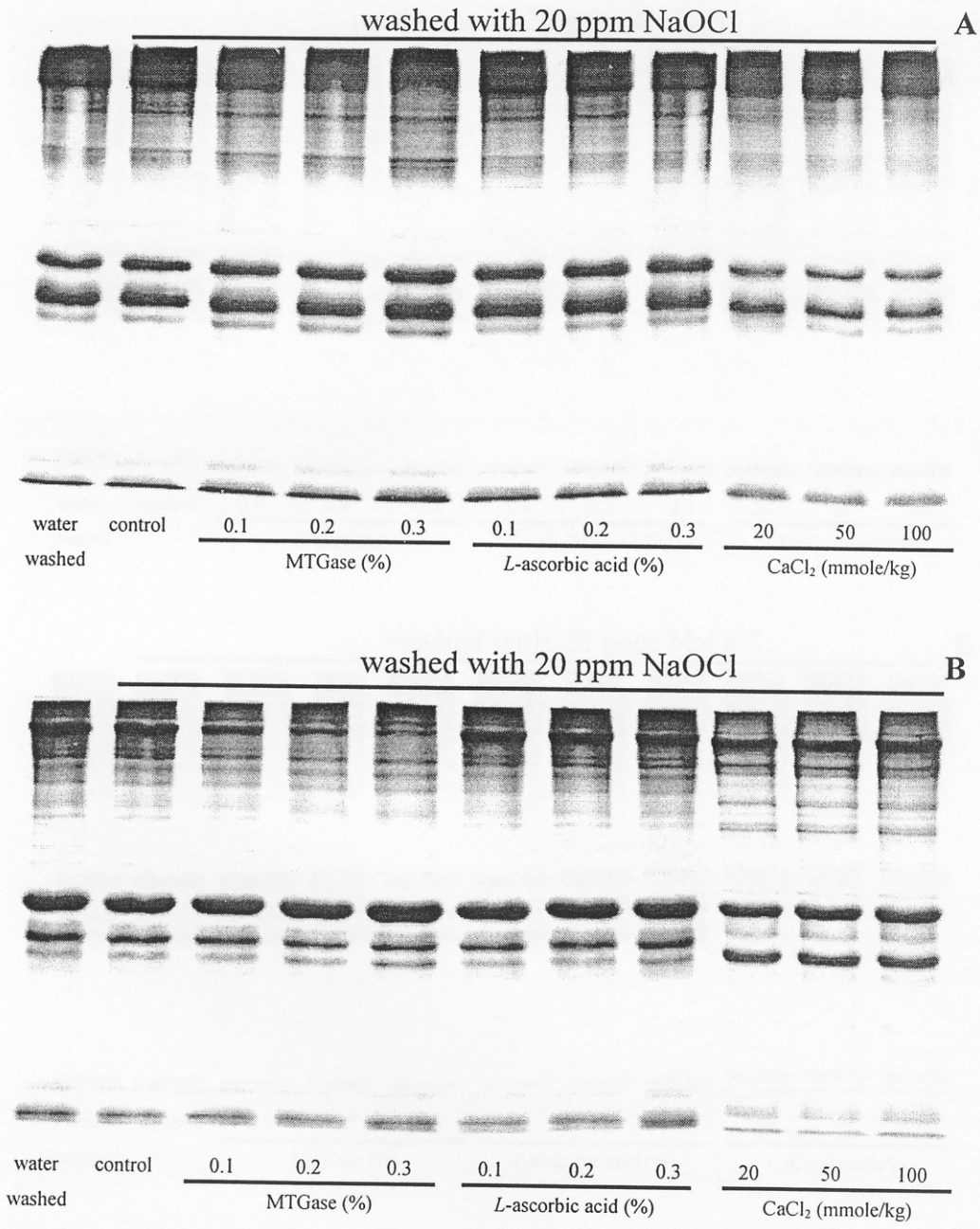

Figure 40 SDS-PAGE pattern of the gel of threadfin bream surimi prepared by 20 ppm NaOCl washing with addition of MTGase, *L*-ascorbic acid or $CaCl_2$. Threadfin bream stored in ice for 14 days was used for surimi preparation. A; non-reducing : B; reducing.

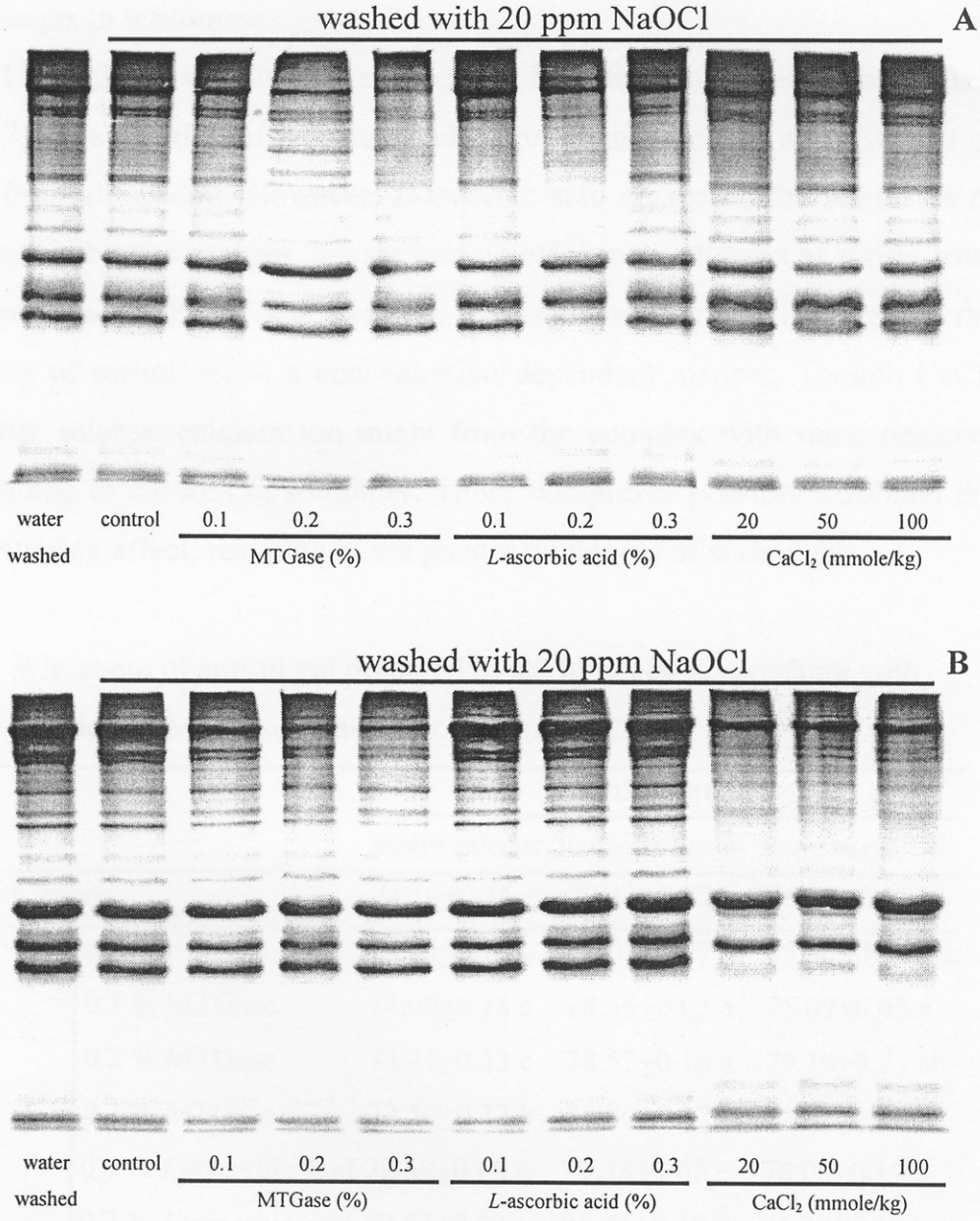

Figure 41 SDS-PAGE pattern of the gel of starry triggerfish surimi prepared by 20 ppm NaOCl washing with addition of MTGase, *L*-ascorbic acid or $CaCl_2$. Starry triggerfish stored in ice for 14 days was used for surimi preparation. A; non-reducing : B; reducing.

3.3 Changes in whiteness

The whiteness of surimi gel produced from threadfin bream was highest (Table 7). The addition of MTGase had no pronounced effect on whiteness of surimi from all species. However, *L*-ascorbic acid decreased the whiteness of surimi gel of bigeye snapper, but showed no effect on whiteness of surimi from other two species. For $CaCl_2$ addition, it was found that $CaCl_2$ increased the whiteness of surimi gel in a concentration dependent manner. Though $CaCl_2$ was water soluble, calcium ion might from the complex with some negative ions, leading to the loss in solubility. Those complexes possibly exhibited the light scattering effect, resulting in the greater whiteness of surimi gels.

Table 7 Whiteness of surimi gel prepared by 20 ppm NaOCl washing with addition of MTGase, *L*-ascorbic acid or $CaCl_2$.

		whiteness		
		bigeye snapper	threadfin bream	starry triggerfish
wash with water		74.19±0.35 c	79.11±0.32 bc	75.42±0.05 bc
washed with 20 ppm NaOCl	control	74.64±0.21 c	80.07±0.07 d	75.32±0.01 abc
	0.1 % MTGase	74.09±0.18 c	78.55±0.03 a	75.09±0.03 a
	0.2 % MTGase	74.21±0.33 c	78.57±0.16 a	75.19±0.23 ab
	0.3 % MTGase	73.50±0.32 b	78.80±0.17 ab	75.52±0.10 c
	0.1 % *L*-ascorbic acid	72.69±0.60 a	79.28±0.02 c	76.00±0.12 d
	0.2 % *L*-ascorbic acid	72.63±0.32 a	80.31±0.30 d	75.38±0.13 bc
	0.3 % *L*-ascorbic acid	72.83±0.46 a	80.10±0.30 d	75.40±0.26 bc
	20 mmole $CaCl_2$/kg	74.38±0.27 c	81.90±0.15 e	76.97±0.19 e
	50 mmole $CaCl_2$/kg	76.01±0.32 d	82.82±0.22 f	78.18±0.13 f
	100 mmole $CaCl_2$/kg	76.42±0.19 d	82.75±0.50 f	79.05±0.14 g

*Values are mean ± standard deviation (n=4). Values with the same letter in the same column are not significantly different ($P>0.05$). Bigeye snapper, threadfin bream and starry triggerfish stored in ice for14 days were used for surimi preparation.

4. Effect of MTGase and chemicals on microstructure of surimi gel

Microstructures of surimi gel from bigeye snapper, threadfin bream and starry triggerfish stored in ice for 14 days without and with the addition of MTGase or chemicals are illustrated in Figure 42, 43 and 44, respectively. Surimi gel from mince washed with 20 ppm NaOCl had finer and longer strands than that from mince washed with water. This suggested that oxidizing agent might induce the cross-linking of protein in the way which increased the protein filaments. These strands could form the network with more fibrillar structure. Among all samples, the gel from bigeye snapper, threadfin bream and starry triggerfish possessed more ordered fibrillar structure when MTGase was added. MTGase was postulated to build up the network structure through intermolecular ε-(γ-glutamyl)lysine cross-linking in co-operation with protein aggregation via hydrophobic interaction, disulfide bonds and/or other interactions during heating process. *L*-ascorbic acid and $CaCl_2$ at the concentration of 0.1 % (w/w) and 50 mmole/kg in surimi from bigeye snapper resulted in the fine and ordered fibrillar structure, suggesting the enhanced cross-linking of muscle protein. From the result, washing mince from three species with NaOCl in combination with MTGase addition could improve the gel-forming ability of low quality fish, while *L*-ascorbic acid or $CaCl_2$ could also improve the gel-forming ability of low quality bigeye snapper.

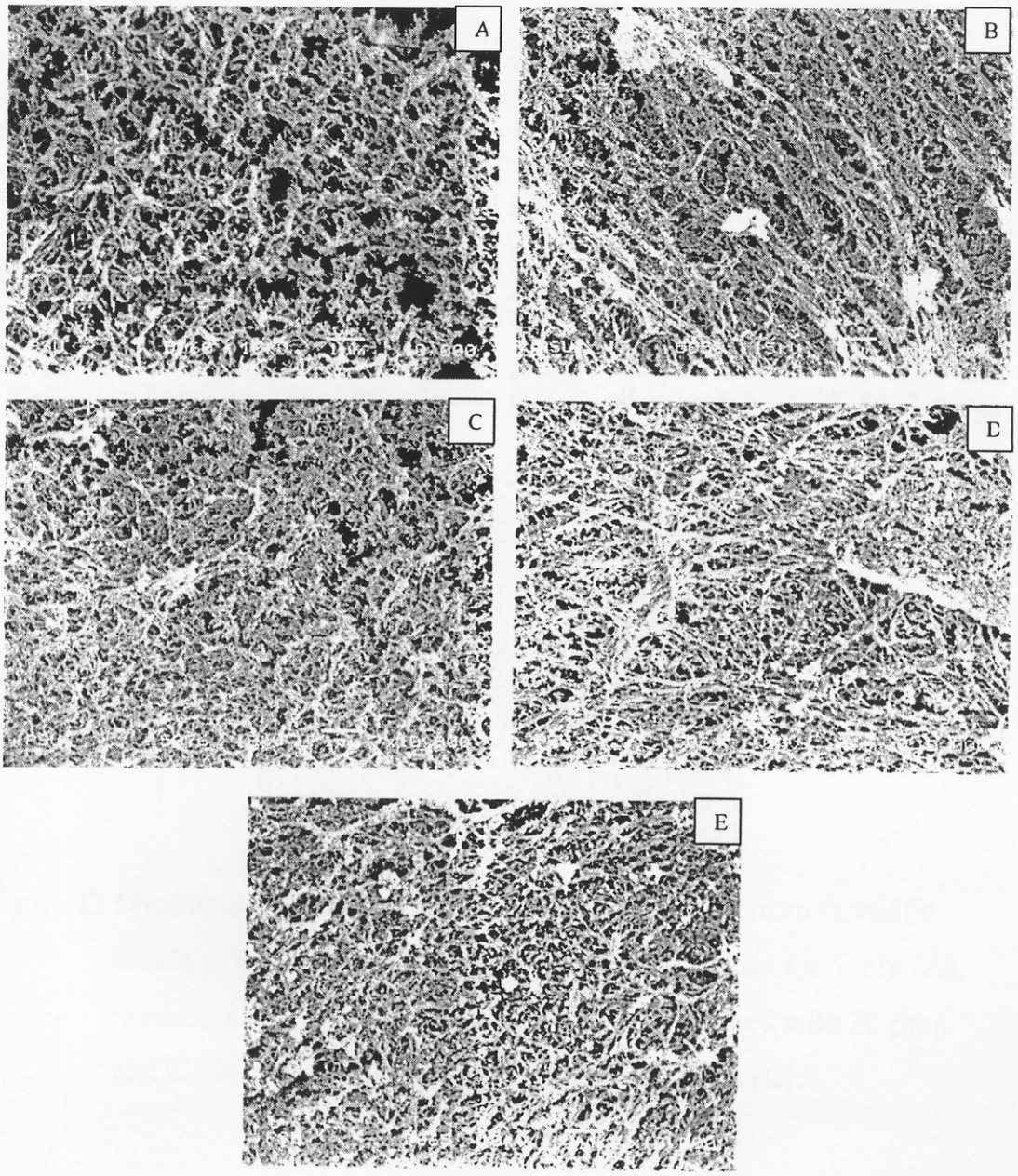

Figure 42 Microstructure of surimi gel (10,000x) prepared from bigeye snapper stored in ice for 14 days, washed with water for 5 min (A), washed with 20 ppm NaOCl for 5 min (B), washed with 20 ppm NaOCl for 5 min and added with 0.2 % MTGase added (C), washed with 20 ppm NaOCl for 5 min and added with 0.1 % *L*-ascorbic acid (D) washed with 20 ppm NaOCl for 5 min and added with 50 mmole $CaCl_2$/kg (E).

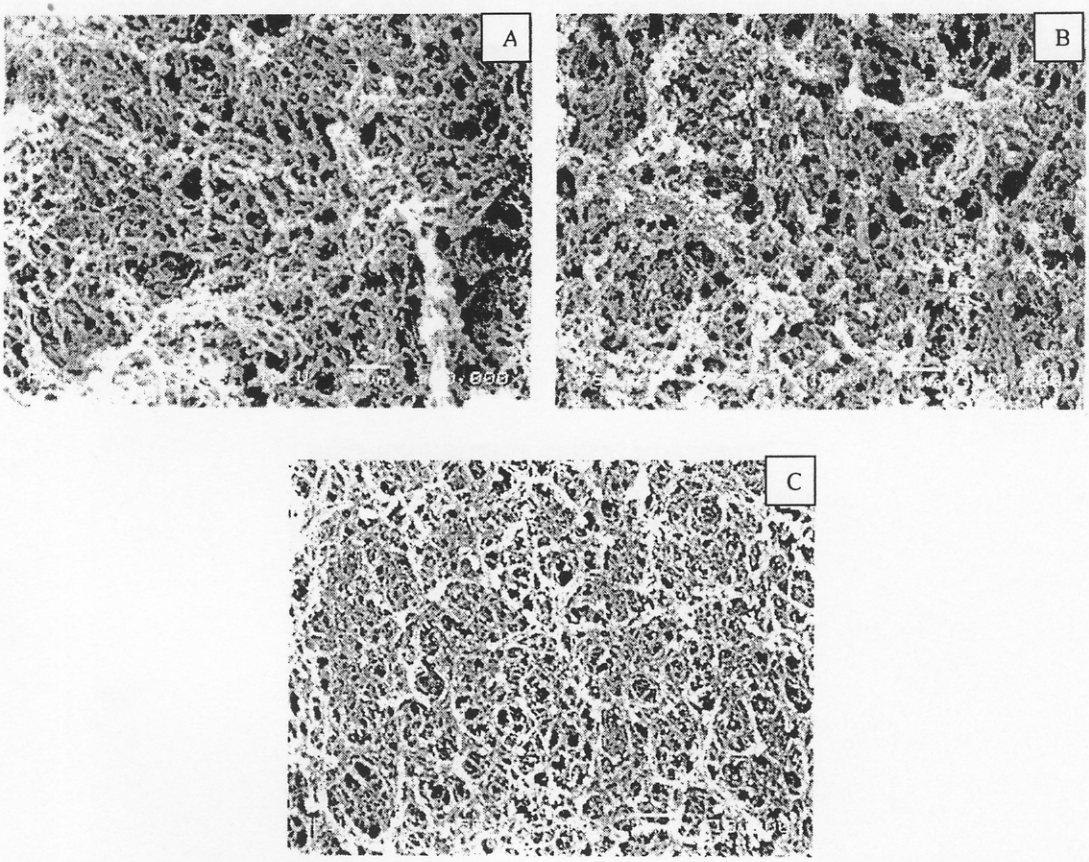

Figure 43 Microstructure of surimi gel (10,000x) prepared from threadfin bream stored in ice for 14 days, washed with water for 5 min (A), washed with 20 ppm NaOCl for 5 min (B), washed with 20 ppm NaOCl for 5 min and added with 0.2 % MTGase (C).

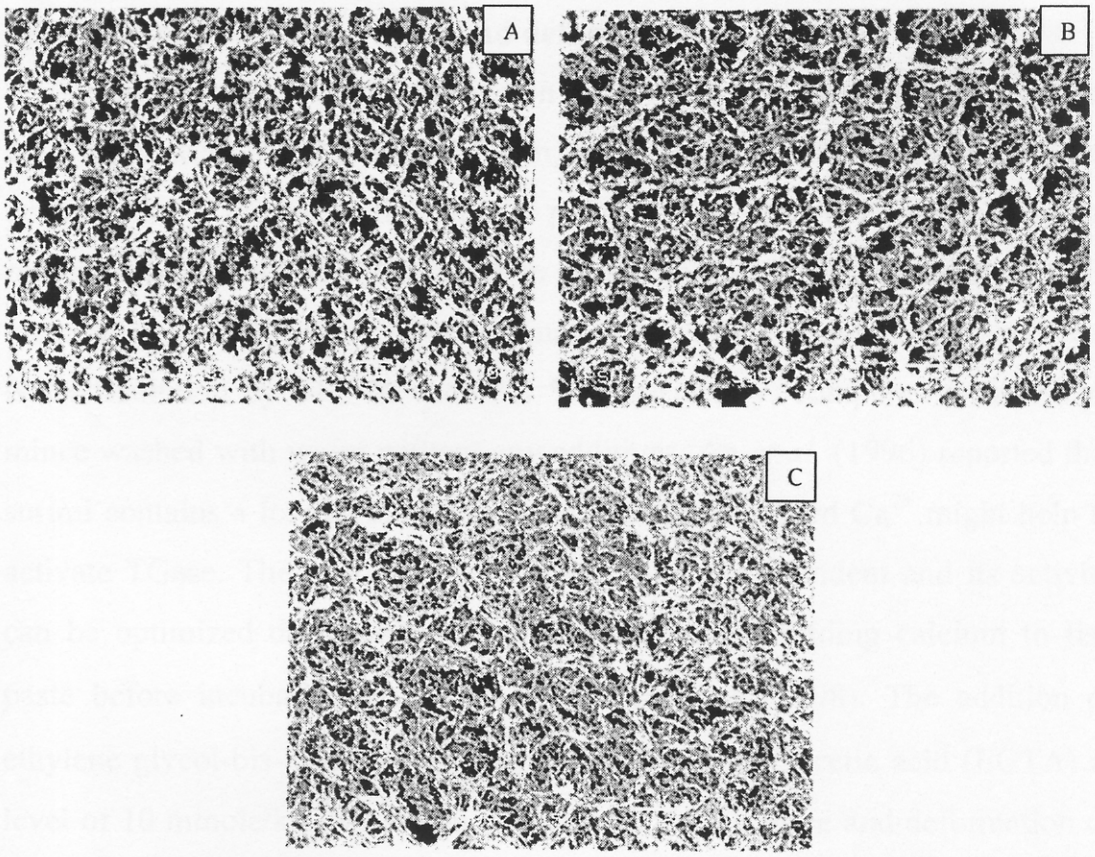

Figure 44 Microstructure of surimi gel (10,000x) prepared from starry triggerfish stored in ice for 14 days, washed with water for 5 min (A), washed with 20 ppm NaOCl for 5 min (B), washed with 20 ppm NaOCl for 5 min and added with 0.2 % MTGase (C).

5. Effect of washing with oxidizing agent on setting of surimi

5.1 Changes in breaking force and deformation

Breaking force and deformation of fresh bigeye snapper mince washed with water were 865.3 g and 11.7 mm, respectively, while breaking force and deformation of mince washed with 20 ppm NaOCl were 902.7 g and 12.1 mm, respectively (Figure 45). The addition of 100 mmole $CaCl_2$/kg resulted in the increase in breaking force of both mince washed with water and washed with NaOCl solution by 14.5 % and 13.4 %, respectively, compared with that of mince washed with water without any additives. An *et al.* (1996) reported that surimi contains a low level of endogenous Ca^{2+} and added Ca^{2+} might help to activate TGase. The endogenous TGase is calcium-dependent and its activity can be optimized during the setting phenomenon by adding calcium to fish paste before incubation at 25-40°C (Lee and Park, 1998). The addition of ethylene glycol-bis-(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA) at level of 10 mmole/kg into surimi decreased breaking force and deformation of mince washed with water by 132.5 % and 87.8 % respectively. EGTA at the same level also decreased breaking force and deformation of mince washed with 20 ppm NaOCl by 120.2 % and 84.7 %, respectively. Therefore, EGTA effectively suppressed the setting as evidenced by the lowered breaking force and deformation. Reduced availability of calcium ion, by the addition of EGTA, caused the lower activity of TGase, which was associated with decreased gel strength. An (1996) reported that the removal of Ca^{2+} from Pacific whiting surimi by EGTA, the Ca^{2+} chelator, resulted in a greatly reduced gel strength even in the presence of 1 % beef plasma protein, owing to the inhibition of endogenous TGase activity. Kumazawa *et al.* (1995) reported that the addition of EDTA resulted in the suppression of ε-(γ-glutamyl) lysine formation. From the result, addition of $CaCl_2$ in surimi washed with 20 ppm NaOCl can increase gel strength. Therefore, washing mince with 20 ppm

NaOCl showed no effect on the activity of endogenous TGase in bigeye snapper surimi.

Breaking force and deformation of surimi prepared from fresh threadfin bream mince washed with water and 20 ppm NaOCl were 360.1 g and 10.1 mm and 400.8 g and 10.5 mm, respectively (Figure 46). The addition of 10 mmole $CaCl_2$/kg in mince washed with water increased breaking force by 6.5 %. However, $CaCl_2$ addition decreased breaking force and deformation of mince washed with 20 ppm NaOCl by 20.3 % and 19.9 %, respectively. The decrease in gel strength might be owing to the denaturation of proteins by $CaCl_2$. Gel added with 10 mmole EGTA/kg had the similar breaking force to gel added with 100 mmole $CaCl_2$/kg, when washed with 20 ppm NaOCl. The result suggested that $CaCl_2$ had no effect on enhanced setting of fresh threadfin bream mince washed with 20 ppm NaOCl. Also, EGTA had no influence on breaking force. Thus, endogenous TGase might be affected by NaOCl washing to some extent. As a consequence, similar breaking force was observed regardless of the presence of $CaCl_2$, activator or EGTA, inhibitor.

Breaking force and deformation of surimi prepared from fresh starry triggerfish mince washed with water and added with 100 mmole $CaCl_2$/kg increased by 28.0 % (Figure 47). Addition of $CaCl_2$ also increased the breaking force of mince washed with NaOCl by 26.0 %, compared with that without $CaCl_2$ addition. Surprisingly, EGTA caused the increase in breaking force of both minces. The EGTA addition in mince washed with water increased breaking force by 48.0 %, compared with the control (washed with water), while breaking force of mince washed with NaOCl increased by 25.8 %, compared with control (washed with water). From the result, EGTA might suppress Ca^{2+}-dependent proteinase, which caused the gel softening. For mince washed with 20 ppm NaOCl, EGTA addition resulted in the same breaking force, in comparison with $CaCl_2$. Therefore, it was suggested that endogenous TGase might not be the important gel enhancer and oxidizing agent might

inactivate the Ca^{2+}-dependent proteinase. Therefore, addition of EGTA could not increase breaking force as it did in mince washed with water. Ca^{2+}-dependent proteinase such as calpain has been reported to be cysteine proteinase. The oxidation of cysteine by oxidizing agent in the active site led to the lowered activity. Therefore, additives play the role in gel strengthening differently among fish species used for surimi preparation.

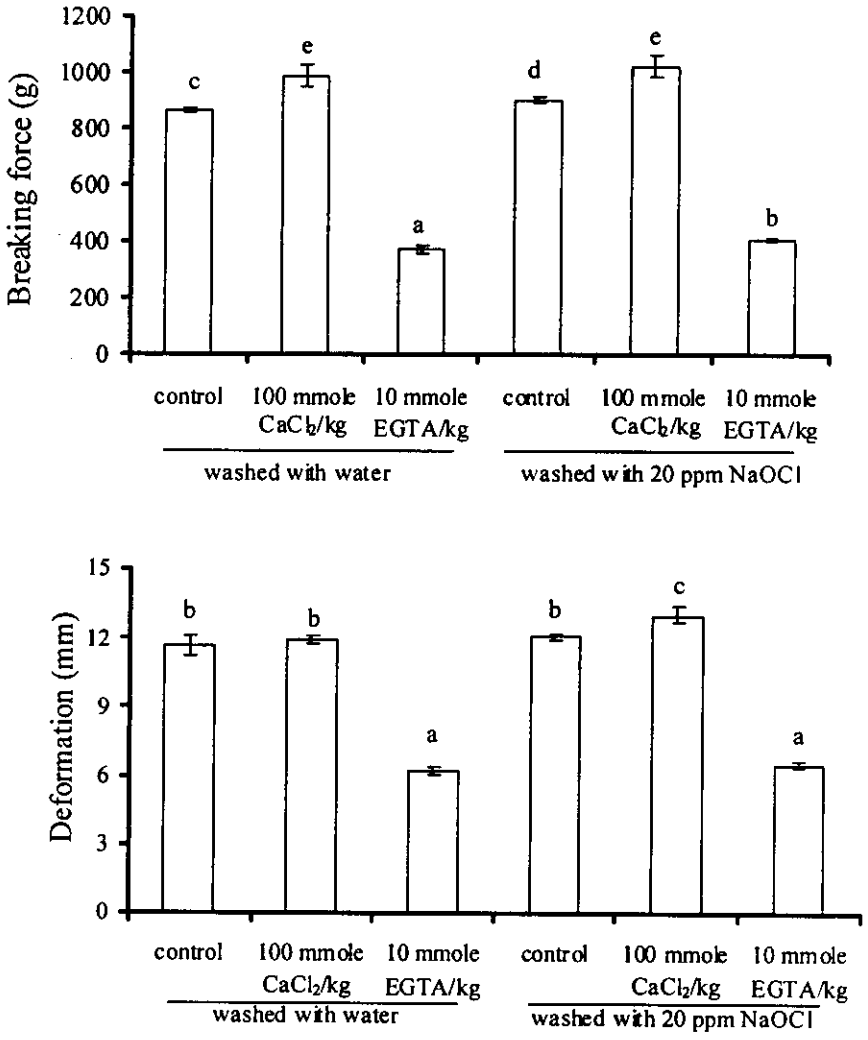

Figure 45 Breaking force and deformation of surimi gel prepared from bigeye snapper added with $CaCl_2$ or EGTA. Bars represent the standard deviation from five determinations.

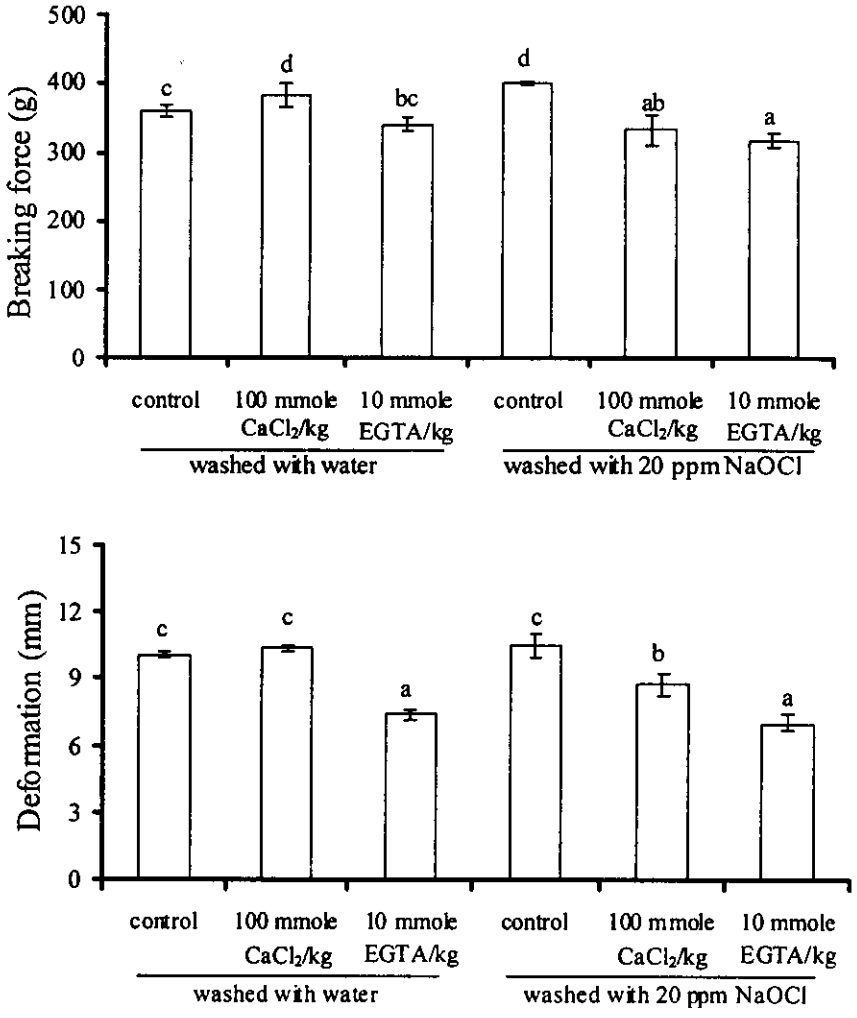

Figure 46 Breaking force and deformation of surimi gel prepared from threadfin bream added with $CaCl_2$ or EGTA. Bars represent the standard deviation from five determinations.

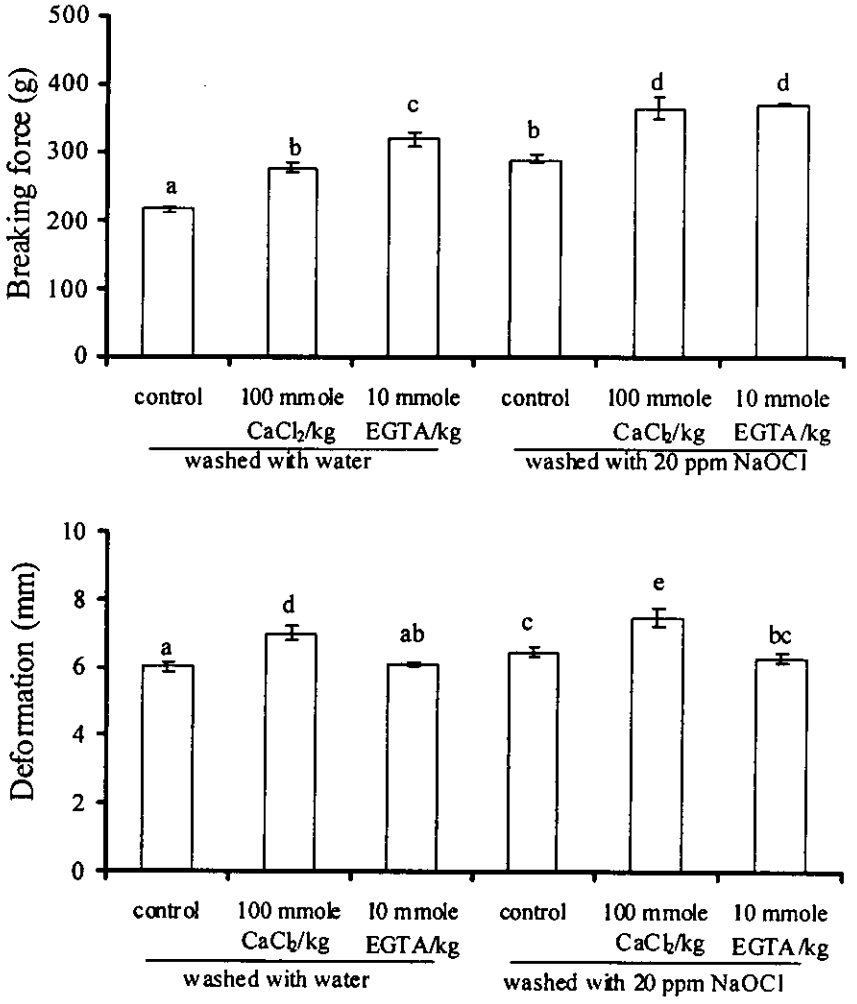

Figure 47 Breaking force and deformation of surimi gel prepared from starry triggerfish added with $CaCl_2$ or EGTA. Bars represent the standard deviation from five determinations.

5.2 Changes in protein pattern of surimi gels

Similar protein patterns of gels from bigeye snapper surimi determined under both reducing and non-reducing conditions were observed (Figure 48, A). It indicated that disulfide bond did not play an essential role in gel formation. $CaCl_2$ at 100 mmole/kg caused the complete disappearance of MHC of the gel, while EGTA resulted in the recovery of MHC. The result suggested that endogenous TGase mainly involved in gel strengthening of bigeye snapper

surimi. For surimi from threadfin bream, it was found that $CaCl_2$ addition had no effect on MHC band intensity under both conditions, reducing and non-reducing (Figure 48, B). However, EGTA addition resulted in the increase in MHC band intensity. From the result, MHC band intensity of gel from surimi with and without $CaCl_2$ addition was similar. It was suggested that Ca ion might not induce endogenous TGase in mince washed with either water or NaOCl. The increase in breaking force with $CaCl_2$ addition (Figure 45-47) might be due to Ca^{2+}-salt bridge mechanism.

For mince washed with NaOCl, Ca^{2+}-salt bridge could not be favored for the oxidized protein. As a result, similar breaking force was observed between mince added with $CaCl_2$ and EGTA (Figure 45). In general, the protein band intensity increased under reducing condition, suggesting that disulfide might involve in stabilizing the gel network of threadfin bream surimi. Similar result was observed between surimi from starry triggerfish and surimi from threadfin bream. For starry triggerfish, though endogenous TGase partially involved in gel strengthening, other factors determining the gel property also affected the gel formation (Figure 48, C). Those factors were influenced by EGTA. However, the mechanism was not well elucidated and need further study.

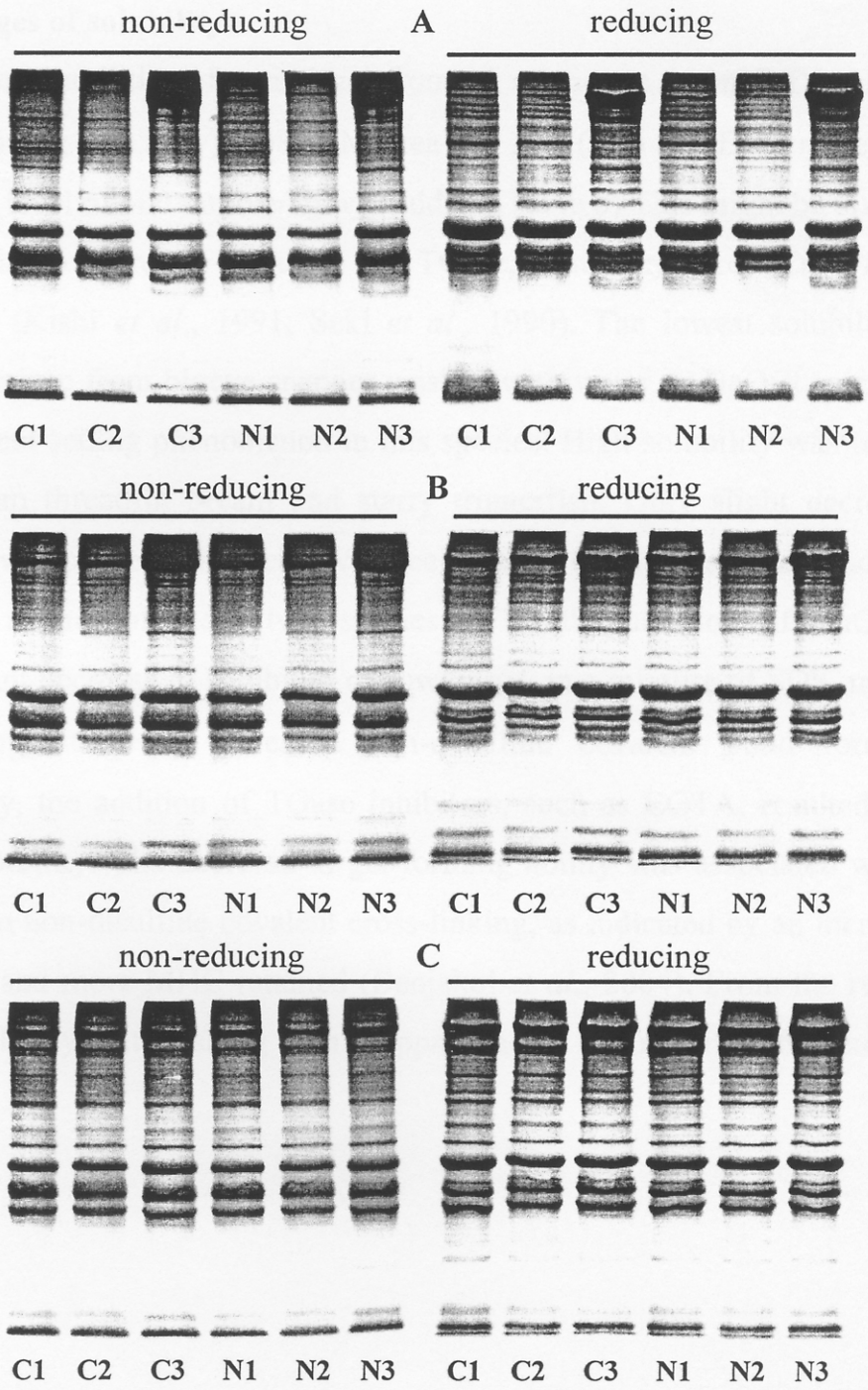

Figure 48 SDS-PAGE pattern of gel surimi from bigeye snapper (A), threadfin bream (B) and starry triggerfish (C).

C and N represent samples washed with water and 20 ppm NaOCl, respectively. Numbers, 1, 2 and 3, represent sample without additive, added with 100 mmole $CaCl_2$/kg and with 10 mmole EGTA/kg, respectively.

5.3 Changes of solubility

The solubilities of surimi gel from all species in 20 mM Tris-HCl, pH 8.0, containing 1 % (w/v) SDS, 8 M urea and 2 % (v/v) β-ME decreased when $CaCl_2$ at a level of 100 mmole/kg was added. (Table 8) This might be due to the cross-linking of protein induced by TGase, which requires Ca^{2+} for full activation (Kishi *et al.*, 1991; Seki *et al.*, 1990). The lowest solubility was found in mince from bigeye snapper washed with water or NaOCl, suggesting the excellent setting phenomenon in this species. High solubility was found in mince from threadfin bream and starry triggerfish. Only slight decrease in solubility was observed in these two species when $CaCl_2$ was added, indicating the poor setting of these two species. With the addition of $CaCl_2$, the concomitant decrease in solubility of suwari gel, in a mixture of SDS, urea and β-ME, suggested the increased non-disulfide covalent bond formation. Conversely, the addition of TGase inhibitors, such as EGTA, resulted in the higher solubility. The decrease in gel-forming ability was associated with the decrease in non-disulfide covalent cross-linking, as indicated by an increase in solubility and more MHC retained (Benjakul *et al.*, 2004). From the result, it was most likely that washing with 20 ppm NaOCl had no effect on activity of TGase.

Table 8 Solubility of surimi gel produced from bigeye snapper, threadfin bream and starry triggerfish with $CaCl_2$ and EGTA addition

Condition		Solubility (%)		
		bigeye snapper	threadfin bream	starry triggerfish
washed with water	Control	44.62±1.53b	78.78±0.39b	86.98±0.13d
	100 mmole $CaCl_2$/kg	41.25±0.17a	76.66±0.37a	84.04±0.56c
	10 mmole EGTA/kg	80.10±2.38c	80.24±0.57b	89.19±0.37e
washed with 20 ppm NaOCl	Control	46.18±0.68b	79.05±0.37b	79.69±0.74b
	100 mmole $CaCl_2$/kg	38.12±0.17a	76.00±0.19a	77.97±0.18a
	10 mmole EGTA/kg	82.20±0.76c	80.90±0.00c	84.30±1.30c

*Values are mean ± standard deviation (n=3). Values with the same letter in the same column are not significantly different (P>0.05).