CHAPTER 3

RESULTS AND DISCUSSION

1. Chemical composition of surimi from tropical fish

Chemical compositions of surimi from three tropical fish are shown in Table 5. All samples contained high moisture content ranging from 75.65 to 78.78%. The surimi contained a high protein content with the negligible fat and ash contents. Protein contents in bigeye snapper, goat fish and threadfin bream surimi were 14.60, 16.16 and 16.05%, respectively. The flesh of fish normally contains 11-24% crude protein, depending on the species, the type of muscle, etc. (Sikorski et al., 1990). During washing process, some lipids, minerals as well as water soluble proteins were removed. As a consequence, the myofibrillar proteins became more concentrated. From the result, carbohydrate at a level of 5.98-7.33% was found in the surimi. Cryoprotectants including sucrose and/or sorbitol are the major sources of carbohydrate found in surimi (MacDonald et al., 2000). Those compounds are used to retard the protein denaturation during freezing or frozen storage (Matsumoto and Noguchi, 1992). TCA soluble peptide content in bigeye snapper, goat fish and threadfin bream surimi varied from 18.35 to 40.61 mmole tyrosine/g sample (Table 5). The result suggested that the degradation occurred at varying extents in different surimi, possibly caused by different freshness of raw material used for surimi production. Lizardfish kept in ice for an extended time showed the higher degradation of muscle protein, especially myosin heavy chain (Benjakul et al., 2002).

Table 5 Proximate compositions of surimi from different tropical fish[#].

Compositions	Bigeye snapper[*]	Goat fish[*]	Threadfin bream[*]
Protein (%)	14.60 ± 0.16[b]**	16.16 ± 0.12[a]	16.05 ± 0.10[a]
Fat (%)	0.04 ± 0.00[c]	0.28 ± 0.00[a]	0.09 ± 0.01[b]
Moisture (%)	78.78 ± 0.03[a]	75.65 ± 0.04[c]	77.19 ± 0.02[b]
Ash (%)	0.60 ± 0.16[a]	0.58 ± 0.00[a]	0.52 ± 0.01[a]
Carbohydrate (%)	5.98 ± 0.29[b]	7.33 ± 0.16[a]	6.15 ± 0.14[b]
TCA soluble peptide (mmole tyrosine/g sample)	18.35 ± 0.16[c]	40.61 ± 0.23[a]	32.71 ± 0.68[b]

*Mean±SD from triplicate determinations.

**The different superscripts in the same row indicate the significant differences ($P<0.05$).
[#]Based on wet basis.

Electrophoretic study of surimi from tropical fish revealed that myosin heavy chain constituted as the major protein, followed by the actin (Figure 4). Surimi is the concentrated myofibrillar protein conventionally prepared by water washing, in which most of sarcoplasmic proteins are removed (Lanier, 2000). As a result, myofibrillar proteins become concentrated. Myosin is the most dominant protein, which constitutes about 50-60% of total myofibrillar protein (Suzuki, 1981). Actin is another protein associated with myosin as actomyosin, which plays an essential role in contraction-relaxation (Trinick, 1991). Due to the filamental nature of myofibrillar proteins, the strong film matrix could be formed (Cuq, 2002). From the result, chemical copositions of surimi from tropical fish were different and varied depending on species. Also, different processes used were postulated for different surimi.

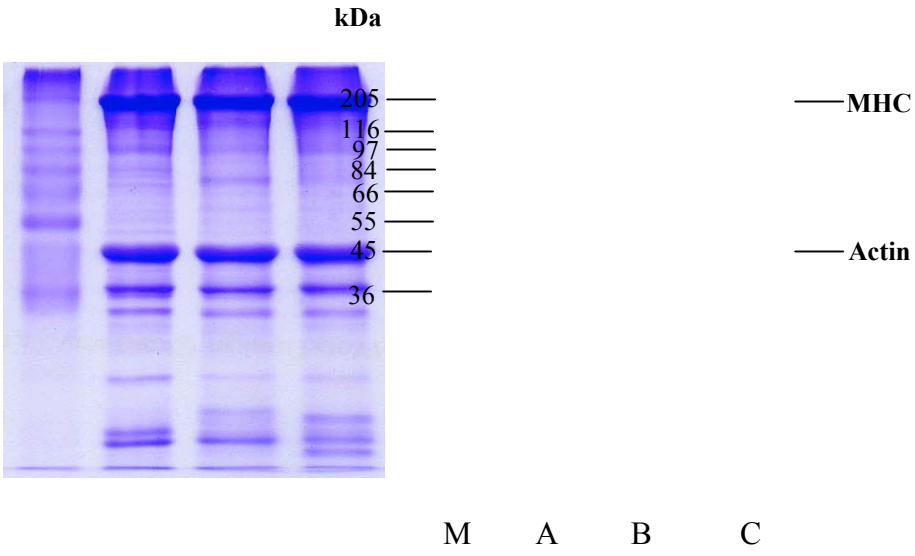


Figure 4 Protein patterns of surimi from different tropical fish. M: high-molecular-weight protein marker; A: bigeye snapper; B: goat fish; C: threadfin bream; MHC: myosin heavy chain.

2. Effect of pH on the properties of film from surimi

2.1 Mechanical property of surimi film

Physical and mechanical properties of surimi from different tropical fish prepared at different pH conditions and protein contents are shown in Table 6. With the same protein content, films from different surimi had the similar thickness. When the same protein content was used, tensile strength (TS) of surimi film prepared from all species at the acidic (pH 3) and alkaline (pH 11) conditions was not different ($P>0.05$). At the same protein content and pH used, bigeye snapper surimi film showed the highest TS, compared with threadfin bream and goat fish surimi films. Nevertheless, elongation at break (EAB) of all surimi films prepared at pH 3 was much higher than that prepared at pH 11 ($P<0.05$) (Table 6). Under the same

condition for film preparation, film from threndfin bream exhibited the lower EAB than did other films ($P<0.05$). However, films from bigeye snapper and goat fish surimi had the similar EAB ($P>0.05$) (Table 6). The unfolded proteins obtained from either acid or alkaline solubilizing process underwent the aggregation through hydrogen, ionic, hydrophobic and covalent bonding, particularly when the water was removed. The degree of chain extension and the nature of sequence of amino acid residues might affect the mechanical properties of protein film (Krochta, 1997). The distribution and extents of inter- and intra-molecular interactions, which give rise to a three-dimentional network structure of the films, could affect their mechanical properties. The main associative forces involved in surimi films was reported to be hydrogen bonds, hydrophobic and ionic interactions (Shiku et al., 2004). Hydrogen bonds are considered important in contributing to the TS of protein films (Meier, 1990). From the study, glycerol was used as the plasticizer to increase film flexibility. This was due to its ability to reduce internal hydrogen bonding between peptide chains while increasing molecular spacing (Lieberman and Gilbert, 1973).

At the same pH used, all surimi films with greater protein content prepared at either pH 3 or 11 exhibited the higher TS ($P<0.05$). Proteins at the higher amount might aggregate intermolecularly to a greater extent, compared with the lower amount, leading to the stronger interaction as evidenced by the increased TS. Nevertheless, no differences in EAB between films with different protein contents were observed, when films were prepared at the same pH. Therefore, different pHs might influence the protein molecules differently, leading to the varying mechanical properties of films, especially EAB. From the result, film network could be somehow affected by pH as evidenced by the different EAB.

Table 6 Physical and mechanical properties of surimi film.

Surimi films	TS* (MPa)	EAB* (%)	WVP** (x 10^{-10} $gm^{-1}s^{-1}Pa^{-1}$)	Thickness** (mm)
Bigeye snapper				
1% pH 3	3.31 ± 0.11^{bc}***	69.19 ± 23.92^{a}	0.90 ± 0.04^{d}	0.018 ± 0.003^{b}
1% pH 11	3.21 ± 0.30^{c}	22.69 ± 13.17^{cd}	0.84 ± 0.03^{de}	0.020 ± 0.004^{b}
2% pH 3	3.89 ± 0.40^{a}	79.96 ± 17.73^{a}	1.17 ± 0.08^{bc}	0.031 ± 0.002^{a}
2% pH 11	3.59 ± 0.30^{ab}	29.27 ± 6.67^{cd}	1.11 ± 0.12^{c}	0.032 ± 0.003^{a}
Goat fish				
1% pH 3	1.29 ± 0.31^{g}	74.14 ± 6.67^{a}	0.86 ± 0.07^{de}	0.019 ± 0.001^{b}
1% pH 11	1.01 ± 0.22^{g}	27.27 ± 3.62^{cd}	0.79 ± 0.05^{e}	0.020 ± 0.001^{b}
2% pH 3	2.19 ± 0.30^{e}	77.97 ± 16.44^{a}	1.22 ± 0.07^{b}	0.031 ± 0.001^{a}
2% pH 11	2.14 ± 0.36^{e}	30.85 ± 9.90^{c}	1.14 ± 0.09^{bc}	0.032 ± 0.002^{a}
Threadfin bream				
1% pH 3	1.80 ± 0.35^{f}	31.31 ± 6.44^{b}	0.87 ± 0.04^{de}	0.019 ± 0.002^{b}
1% pH 11	1.79 ± 0.16^{f}	16.47 ± 4.25^{d}	0.82 ± 0.01^{de}	0.021 ± 0.003^{b}
2% pH 3	2.85 ± 0.39^{d}	49.86 ± 7.30^{b}	1.53 ± 0.08^{a}	0.031 ± 0.002^{a}
2% pH 11	2.62 ± 0.41^{d}	23.52 ± 8.61^{cd}	1.49 ± 0.06^{a}	0.032 ± 0.002^{a}

*Mean±SD from eight determinations.
**Mean±SD from five determinations.
***The different superscripts in the same column indicate the significant differences ($P < 0.05$).

2.2 Water vapor permeability of surimi films

Water vapor permeability (WVP) of different surimi films prepared at the acidic and alkaline pHs with different protein contents is shown in Table 6. At the same protein content used, no differences were found between surimi films prepared at pH 3 and 11 ($P>0.05$). Threadfin bream surimi films with 2% protein content had the highest WVP, compared with other surimi films ($P<0.05$). WVP of surimi films prepared at both pHs increased with increasing protein contents ($P<0.05$). A higher amount of protein was probably associated with a higher amount of polar groups in surimi film, which could absorb more water from the surrounding atmosphere. Blue marlin myofibrillar proteins comprised a large amount of ionized polar amino acids (approximately 33%) (Shiku et al., 2003). Increased transmission of water vapor through protein-based film is also caused by the presence of glycerol, a hydrophilic plasticizer (Cuq et al., 1995). Sucrose and sorbitol used as cryoprotectants also provided the polar groups in the surimi films, where hydrogen bonding can be formed with water (Kim and Ustunol, 2001). When the greater surimi amount was used, the concomitant increase in cryoprotectant amount was obtained, leading to the greater WVP. WVP of myofibrillar protein-based biopackaging was much greater than those of typical polymeric packaging materials such as low density and high density polyethylene films (Cuq et al., 1995).

2.3 Color and transparency

L*, a* and b*-values of bigeye snapper, goat fish and threadfin bream surimi films prepared at different pHs and protein contents are shown in Table 7. At

1% protein content, no differences in a* and b*-values were observed, but L*-value of films prepared at pH 11 was greater ($P<0.05$). However, at 2% protein content, film from bigeye snapper and goat fish surimi prepared at pH 3 had the greater b*-value but lower L*-value ($P<0.05$). For threadfin bream surimi film, pHs did not show any effect on L*, a* and b*-values of film prepared using 2% protein. The result suggested that the surimi films prepared at acidic pH was more likely yellowish than those prepared at alkaline pH as evidenced by greater b*-value. Film prepared at pH 3 had the lower lightness than that prepared at pH 11. From the result, it indicated that acidic condition might induce the formation of yellowish pigment, especially via Maillard reaction. Acidic condition probably induced the hydrolysis of protein as well as sugar, leading to the availability of amino acid group and carbonyl group from reducing sugar. As a consequence, Maillard reaction was favored, particularly during drying of film. Maillard reaction is a type of non-enzymatic browning reaction. It results from an initial reaction of a reducing sugar with an amino compound, followed by a cascade of consecutive and parallel reactions to form a variety of colors (Martins and Van, 2005). At the same pH used, protein content affected the color differently. The greater b*-value with lower L*- and a*-values were generally noticeable with films having 2% protein content, compared with those containing the lower protein content (1%) ($P>0.05$), except for threadfin bream surimi film. Thus, the films with 2% protein content had more yellowness but lower lightness than those having 1% protein content. The increase in surimi used resulted in the increase in free amino groups as well as the carbonyl groups from cryoprotectant, especially under acidic condition. Thus, the browning was enhanced via Maillard reaction as shown by the increased yellowness (b*-value).

The transparency of bigeye snapper, goat fish and threadfin bream surimi films prepared at different pHs and protein contents is shown in Table 7. The film was more transparent when the lower protein content was used ($P<0.05$). No differences in transparency were observed between films prepared at different pHs, when the same protein content was used ($P>0.05$). Among all film tested, film from goat fish surimi possessed the highest transparency value, compared with other surimi films. The results indicated that film from goat fish surimi were less transparent than those from other surimi. The greater protein content might favor the aggregation of protein molecules in the film network. Also, the higher amount of protein possibly retarded the light transmission of the film. This led to the decrease in transparency when the greater protein content was used. At the same protein content used (1%), films from bigeye snapper surimi was more transparent (4.29-4.33) than those from blue marlin meat (8.02-8.34) reported by Shiku et al. (2003).

Table 7 L*, a* and b*-values of surimi film.

Surimi films	L*#	a*#	b*#	Transparency#
Bigeye snapper				
1% pH 3	90.41 ± 0.07^{gh}*	-1.23 ± 0.10^{a}	1.44 ± 0.04^{ef}	4.33 ± 0.12^{e}
1% pH 11	91.17 ± 0.10^{b}	-1.29 ± 0.16^{a}	1.09 ± 0.05^{f}	4.29 ± 0.08^{e}
2% pH 3	90.23 ± 0.04^{i}	-1.70 ± 0.07^{c}	3.76 ± 0.56^{b}	5.93 ± 0.19^{bc}
2% pH 11	90.93 ± 0.02^{d}	-1.55 ± 0.13^{bc}	1.45 ± 0.38^{ef}	5.84 ± 0.25^{c}
Goat fish				
1% pH 3	90.78 ± 0.15^{e}	-1.57 ± 0.06^{bc}	2.60 ± 0.57^{d}	5.38 ± 0.46^{d}
1% pH 11	90.97 ± 0.04^{d}	-1.56 ± 0.05^{bc}	2.28 ± 0.15^{d}	5.36 ± 0.38^{d}
2% pH 3	90.37 ± 0.02^{h}	-1.56 ± 0.13^{bc}	4.25 ± 0.07^{a}	6.43 ± 0.05^{a}
2% pH 11	90.47 ± 0.15^{g}	-1.58 ± 0.08^{bc}	3.07 ± 0.03^{c}	6.36 ± 0.31^{ab}
Threadfin bream				
1% pH 3	91.08 ± 0.01^{c}	-1.20 ± 0.06^{a}	1.72 ± 0.08^{g}	4.54 ± 0.38^{e}
1% pH 11	91.88 ± 0.06^{a}	-1.28 ± 0.07^{a}	1.65 ± 0.05^{g}	4.54 ± 0.24^{e}
2% pH 3	90.63 ± 0.04^{f}	-1.46 ± 0.03^{b}	1.46 ± 0.01^{e}	5.21 ± 0.19^{d}
2% pH 11	90.68 ± 0.03^{f}	-1.48 ± 0.11^{b}	1.28 ± 0.03^{ef}	5.15 ± 0.05^{d}

#Mean±SD from three determinations
*The different superscripts in the same column indicate the significant differences ($P < 0.05$).

2.4 SDS-PAGE patterns of surimi films

Protein patterns of films from different surimi prepared at the acidic and alkaline conditions are depicted in Figure 5. No MHC band was found in films from all species under both acidic and alkaline conditions. All surimi films also contained the lower band intensity of actin, compared with those of surimi (Figure 4).

Those changes were observed with the concomitant appearance of proteins with the lower molecular weight. This suggested that the degradation of proteins, especially MHC, occurred in the films. From the result, different protein patterns were found between films prepared at pH 3 and 11. The degraded proteins with molecular weight of 130-150 kDa were found in bigeye snapper, threadfin bream and goat fish surimi films with acid solubilizing process. Surimi films from bigeye snapper, goat fish and threadfin bream surimi with alkaline solubilizing process had no protein bands with MW of 120 and 50 kDa as found in films prepared with acid solubilizing process. Only protein band with MW of 150 kDa was found in film from bigeye snapper surimi with alkaline solubilizing process. Therefore, the cleavage of myosin might be taken place at different sites, leading to the differences in the molecular distribution. Actin was also degraded into different degradation products, especially by alkaline solubilizing processes. Therefore, the acid or alkaline solubilizing process might induce the degradation of muscle protein in surimi. This possibly contributed to the different characteristics, both mechanical properties (Table 6) and color (Table 7) of surimi films produced by both processes. Cuq et al. (1995) found the degradation of myosin heavy chain in edible films based on sardine myofibrillar proteins, especially in the acidic pH ranges due to the cathepsins. However, no degradation of myosin heavy chain was found in the film from Alaska pollack surimi (Shiku et al., 2004). Owing to different enzymes in muscle of various fish (Haard et al., 1994), the varying degree of hydrolysis in the films based on muscle protein from different fish species was postulated.

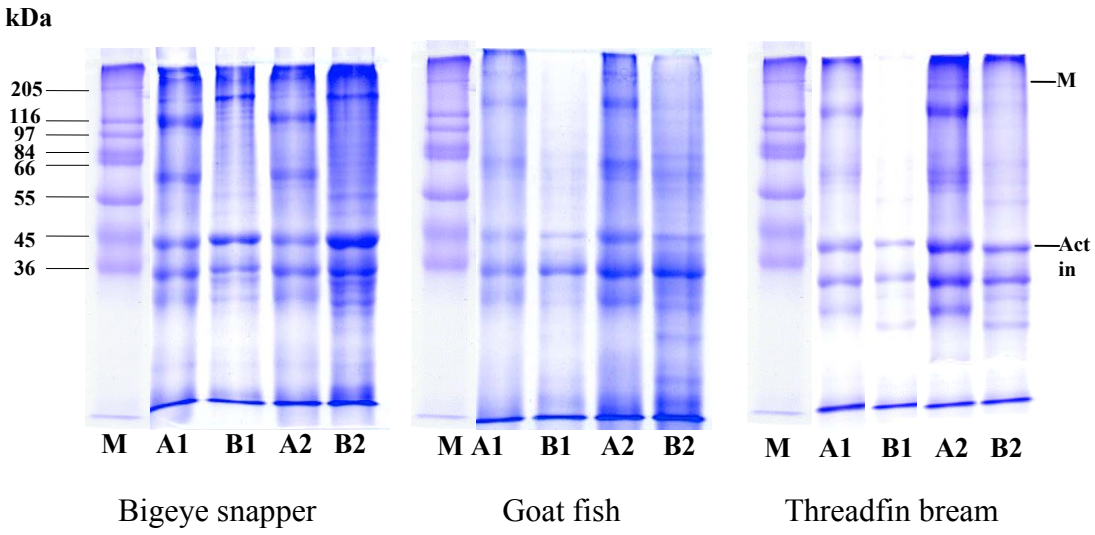


Figure 5 Protein patterns of surimi film from bigeye snapper, goat fish and threadfin bream. M: high-molecular-weight protein marker; A1: 1% protein content, pH 3; B1: 1% protein content, pH 11; A2: 2% protein content, pH 3; B2: 2% protein content, pH 11.

2.5 Film solubility and protein solubility of surimi film

Table 8 shows water solubility and protein solubility of bigeye snapper, goat fish and threadfin bream surimi films. At the same protein content used, all surimi films prepared at pH 3 had the lower water solubility and protein solubility than those prepared at pH 11. The result suggested that the film with alkaline solubilizing process had the lower cross-linking with the weaker bonding, which was possibly associated with the shorter chain length of protein molecules (Figure 5). As a result, the interaction between proteins could be lowered. This resulted in the higher solubility of resulting film. At the same pH used, all surimi films with greater protein content exhibited the higher water solubility and protein solubility ($P<0.05$).

The greater concentration of protein used for film-forming solutions might affect the film formation, particularly the bonding or aggregation of protein molecules. With the greater content of protein, the removal of water could be lower than that found in the film-forming solution with lower protein content. This might cause the more intensive cross-linking of adjacent protein molecules of film-forming solution with the lower protein content. As a result, both water solubility and protein solubility of those films with the lower protein content were lower than those with the greater protein content. Myofibrillar protein-based films are substantially influenced by plasticization from incorporated plasticizers (e.g., glycerol, sorbitol, and sucrose) and from moisture (Cuq et al., 1995; 1997). In general, hydrophilic plasticizers such as glycerol enhanced water solubility (Cuq, 2002). Cuq (2002) reported that increasing the plasticizer content in the film increased the water-soluble dry content. The larger solubility of surimi films probably described the weaker structure of the films (Shiku et al., 2004). From the result, the greater water solubility was observed, compared with protein solubility. This was likely due to the solubilization of glycerol in the film into water.

From the result, protein solubility of films prepared under alkaline condition was higher than that of film prepared with acidic condition. This was probably because the ease of lower MW protein molecules to be leached out. The more degradation was found in the film with alkaline solubilization process (Figure 5).

Table 8 Film solubility and protein solubility of surimi film.

Surimi films	Film solubility[#] (%)	Protein solubility[#] (%)
Bigeye snapper		
1% pH 3	43.29 ± 1.21[f*]	17.83 ± 0.13[g]
1% pH 11	51.65 ± 1.02[e]	33.29 ± 3.72[c]
2% pH 3	67.85 ± 0.31[d]	24.74 ± 0.40[de]
2% pH 11	72.01 ± 0.88[b]	39.37 ± 3.77[b]
Goat fish		
1% pH 3	44.69 ± 0.44[f]	19.47 ± 0.63[fg]
1% pH 11	52.76 ± 1.82[e]	38.49 ± 0.85[b]
2% pH 3	69.66 ± 0.29[c]	27.03 ± 2.40[d]
2% pH 11	74.08 ± 0.99[a]	43.76 ± 0.48[a]
Threadfin bream		
1% pH 3	43.52 ± 0.42[f]	19.07 ± 0.96[fg]
1% pH 11	53.15 ± 0.57[e]	34.31 ± 4.93[c]
2% pH 3	68.73 ± 0.69[cd]	22.45 ± 0.63[ef]
2% pH 11	73.34 ± 0.48[ab]	41.13 ± 2.11[ab]

#Mean±SD from three determinations
*The different superscripts in the same column indicate the significant differences ($P < 0.05$).

From the result, bigeye snapper surimi films with 2% protein content at the acidic condition had the highest mechanical property and lowest WVP, compared

with other samples. Therefore, bigeye snapper surimi films with 2% protein content prepared at the acidic pH was chosen and used for further study.

3. Compositional changes of surimi film-forming solution as affected by acidic and alkaline pHs

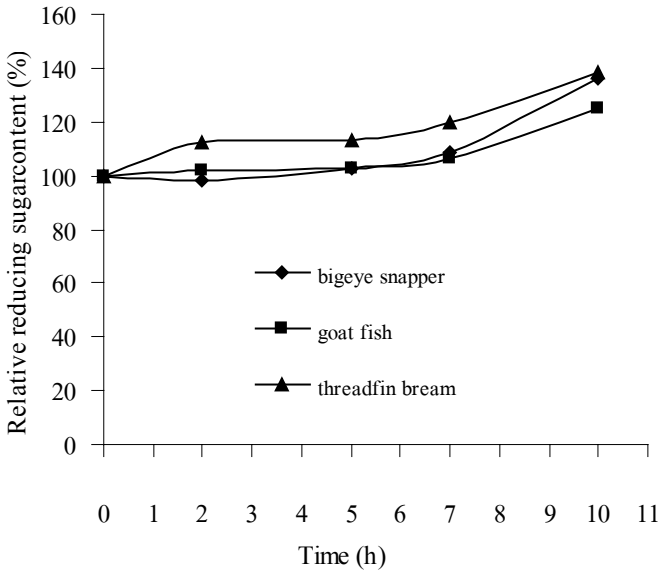
To further investigate the effect of pH on color of surimi film, the change of reducing sugar content and protein patterns were monitored in surimi film-forming solutions with different pHs.

3.1. Reducing sugar content

The reducing sugar content of acidic and alkaline film-forming solutions is depicted in Figure 6. The reducing sugar content of film-forming solutions of bigeye snapper, goat fish and threadfin bream surimi with pH 3 and 11 increased as the exposure time increased up to 10 h ($P<0.05$). However, the rate of increase was much greater in the acidic solution, particularly with increasing exposure time. Acidic condition might induce the hydrolysis of sucrose used as the cryoprotectant in surimi, resulting in the increased reducing sugar released, both glucose and fructose. Acid effectively hydrolyzes disaccharide to monosaccharide. Generally, hydrolysis of glycosidic bonds joining monosaccharide (glycosyl) units in di-and polysaccharides can be catalyzed by either acids or enzymes (BeBiller and Whistler, 1996; Cierezko et al., 1998). As a consequence, those reducing sugars might react with free amino groups of degraded proteins in surimi via Maillard reaction, especially during drying. Brown or yellowish compounds were formed via this reaction (Wong, 1989). Since the reducing sugar content in acidic film-forming solutions was higher than alkaline film-forming solutions, browning of the surimi

films prepared by the former solution was greater than the latter as shown by the higher b*-value in the films prepared at acidic condition (Table 7).

(A)



(B)

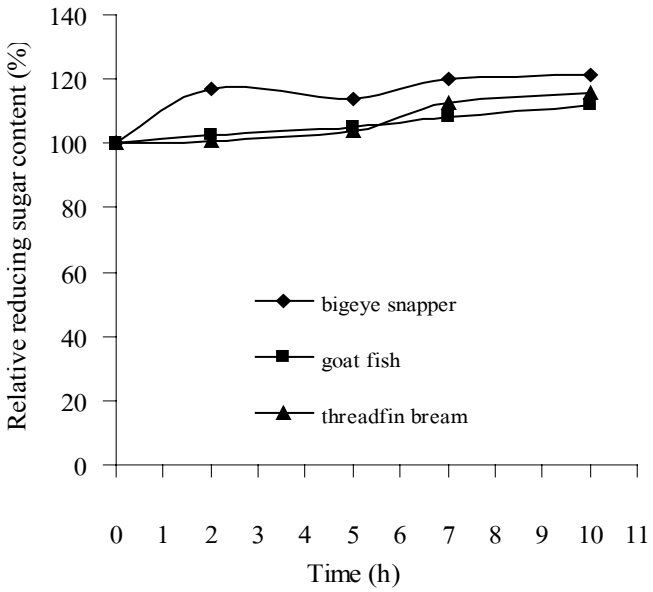


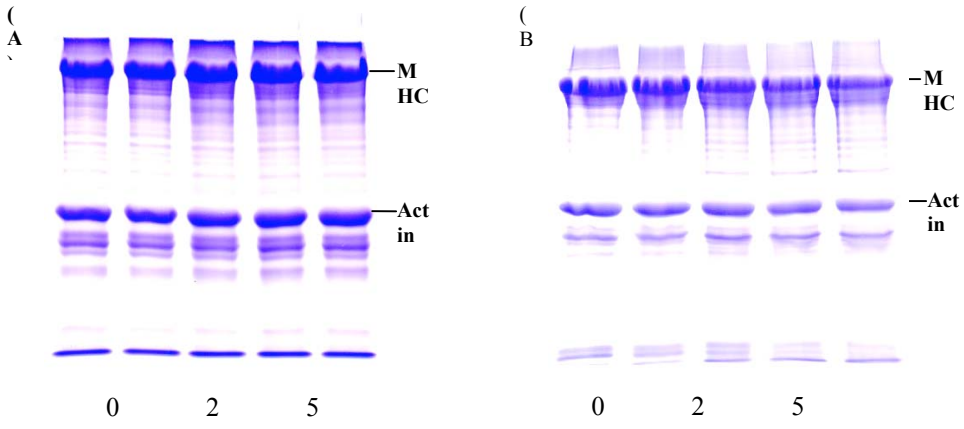
Figure 6 Reducing sugar content of acidic (A) and alkaline (B) film-forming solutions of surimi from different tropical fish with different exposure times.

3.2. SDS-PAGE patterns of bigeye snapper, goat fish and threadfin bream surimi film-forming solution

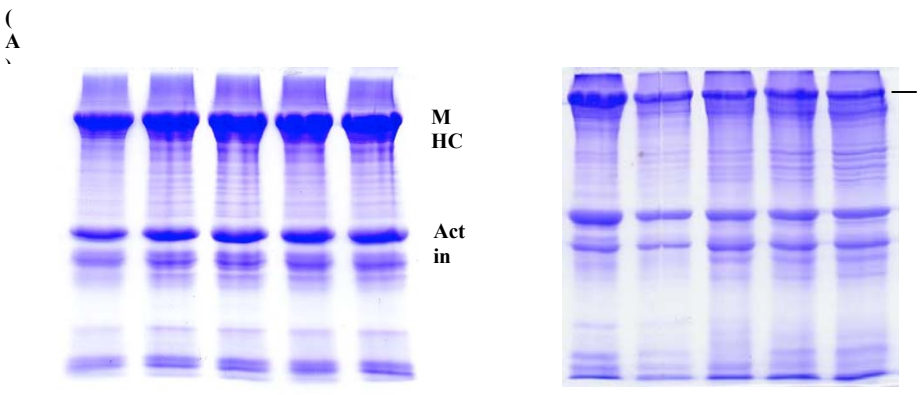
The protein patterns of acidic and alkaline film-forming solutions of bigeye snapper, goat fish and threadfin bream surimi are shown in Figure 7. At 0 h, the protein patterns of film-forming solution from bigeye snapper, goat fish and threadfin bream surimi were similar to those of surimi. For alkaline solution, MHC was more degraded as the exposure time increased up to 10 h. Actin was also hydrolyzed with increasing exposure time under alkaline condition. Generally, MHC was more susceptible to degradation than actin. Benjakul et al. (2003) also reported that MHC was more degraded than actin in bigeye snapper during iced storage. At acidic condition, negligible degradation was observed throughout 10 h. The result suggested that autolysis caused by endogenous proteases was greater at alkaline pH.

This might be due to the greater proteolytic activity of alkaline protease in all surimi used, particularly myofibril bound protease. As a consequence, the degradation could be enhanced by those proteases under alkaline pH as evidenced by the greater hydrolysis of MHC (Figure 7). Bigeye snapper muscle was reported to contain the heat-stable alkaline proteinase, which could degrade MHC effectively (Benjakul et al., 2003a; Benjakul et al., 2003b). However, Saunders (1994) reported the degradation of myofibrillar proteins treated at acidic pH for 20 h by the action of acid-proteinase (mainly cathepsin). Chawla et al. (1996) reported the degradation of myosin heavy chain of acid treated threadfin bream mince, due to the presence of acid protease. From the result, the degradation took place to a higher extent at alkaline pH, leading to the decreased peptide chain length. This might be associated with the lower EAB in all surimi film prepared at alkaline pH (Table 6). The differences between protein patterns in film-forming solution and surimi film (Figure 5) might be due to the differences in acid and alkaline concentrations. During drying, water was removed and acid or alkaline became more concentrated. Those drastic conditions resulted in the enhanced hydrolysis under both pH conditions as evidenced by no MHC remained in the films (Figure 5). Thus, pH conditions affected the protein degradation in film-forming solution, especially during drying. Those protein changes most likely determined the resulting film properties.

Bigeye snapper surimi



Goat fish surimi



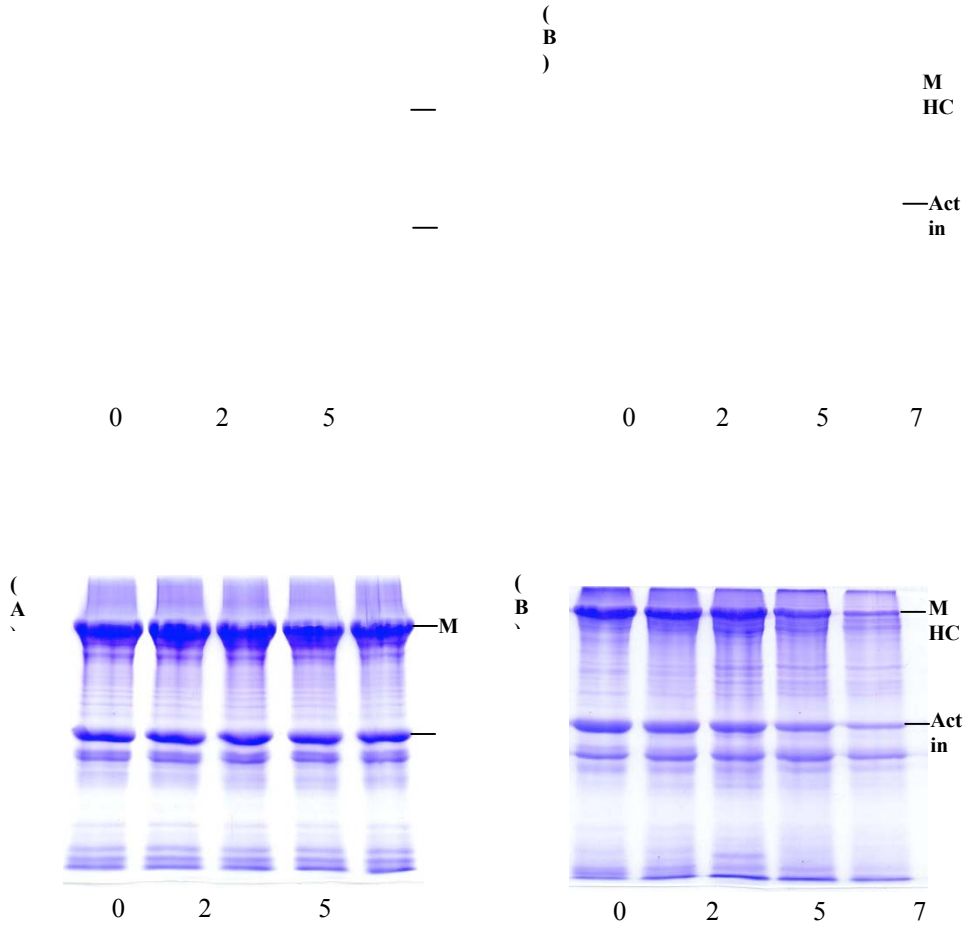


Figure 7 Protein patterns of acidic (A) and alkaline (B) film-forming solutions from bigeye snapper surimi, goat fish surimi and threadfin bream surimi with different exposure times. MHC: myosin heavy chain. The numbers designate the exposure time (h).

4. Effect of lipid types and concentrations on the properties of bigeye snapper surimi film

4.1 Mechanical properties of bigeye snapper surimi film incorporated with lipid

Films with different types and amounts of lipids had the similar thickness (Table 9). TS of bigeye snapper surimi film added with different lipids at various concentrations is shown in Table 9. In general, the addition of lipids (25-75% glycerol substitution) to surimi films reduced their TS ($P<0.05$) due to the partial replacement of protein polymer by lipids in the film matrix. The interactions between non-polar lipid molecules and between polar polymer and non-polar lipid molecules are believed to be much lower than those between the polar polymer molecules (Yang and Paulson, 2000). With 25% glycerol substitution, the lowest TS was observed in all films added with any type of lipids. Strength reduction of edible composite films with lipid incorporation has been typically reported (Debeaufort and Voilley, 1995; Shellhammer and Krochta, 1997; Yang and Paulson, 2000; Morillon et al., 2002; Bertan et al., 2005). However, TS of the composite film generally increased as the lipid levels used to substitute the glycerol increased up to 100% ($P<0.05$). The highest TS was observed with 100% glycerol substitution. The result suggested that the addition of a greater amount of lipid into film-forming solution resulted in the less incorporation of lipids in dispersed two-phase system even though Tween-20, a non-ionic surfactant, was used to stabilize the emulsion of the system. Prior to film casting, surimi proteins were solubilized by adjusting the pH to the acidic range. Charge repulsion contributes to the protein solubility, which is prerequisite for film formation. Unfolding of protein molecules at low or high pH values occurs owing to a decrease in electrostatic bonds (Vojdani, 1996). As a consequence, the lipids added

could not be compatibly incorporated with the charged protein molecules in the solution. Without the aid of Tween-20, the added lipids were totally separated from the protein-rich phase and located on the top of the film. Additionally, in the presence of the less amount of glycerol, a hydrophilic plasticizer, with the concomitant increasing amount of lipids, the higher aggregation of protein molecules in the protein-rich phase of the film was presumed. This led to the increased TS, especially film without glycerol. However, film containing 100% palm oil as the plasticizer (100% glycerol substitution) had the similar TS to the control (100% glycerol). For the sample added with solid lipids at 100% glycerol substitution, TS was found to be varied with lipid types. It was noted that film containing 100% shortening had the greater TS than that consisting of 100% butter. Butter has the discontinuous structure of fat globules and a crystalline fat matrix, while shortening possesses the plate-like three dimensional crystalline network with crystal bridge (Juriaanse and Heertje, 1988). Fat globule more remaining in the butter structure could partially contribute as the plasticizer of film as evidenced by the lower TS (Table 9). This observation was inconsistent to some previous literatures concerning whey protein/lipid composite films reported by Gontard et al. (1994) and Shellhammer and Krochta (1997), and gelatin/lipid composite films reported by Bertan et al. (2005). The differences were most likely due to the differences in protein structure and protein solubilizing process used for the preparation of film-forming solution. The results suggested that the strength of surimi protein/lipid composite films was governed not only by the nature of lipid phase but also by molecular interactions (aggregation) in the protein-rich phase.

When EAB of films added with different lipids at varying levels of glycerol substitution was determined (Table 9), it was noted that EAB of films added with lipids at 25% glycerol substitution showed the similar EAB to the control. However, EAB increased gradually when the palm oil was added up to 75% glycerol substitution. For the samples added with butter or shortening, the slight decrease in EAB was noticeable when the lipids were added up to 75% glycerol substitution. The differences in changing pattern of EAB between films added with liquid or solid lipids were most likely due to the differences in their molecular flexibility and phase solidity. The liquid lipid could be dispersed in the protein-rich phase more effectively than the solid lipids and possibly exhibited more potentially plasticizing effect. Similar results have been reported for whey protein films (Anker et al., 2002), zein films (Lai and Padua, 1998) and egg albumen films (Gennadios et al., 1996). Conversely, the solid lipid became solidified and larger phase separation occurred upon film formation. The non-uniform system might be associated with the lack of film structural integrity. Martin-Polo (1992) observed that film incorporated with solid lipid exhibited heterogeneous and porous structure while film incorporated with liquid lipid was denser in structure. The lower continuity and coherence (cohesiveness) of protein network in the presence of lipid globules might result in the decrease in EAB as pointed out by Anker et al. (2002) and Péroval et al. (2002). Lower water content of film containing lipids may also cause the decreased elongation (Gallo et al., 2000).

At level of 100% glycerol substitution, EAB of all samples sharply decreased and reached the values ranging from 1.26 to 2.47%. The excessive protein aggregation occurred in the absence of water soluble plasticizer resulted in the

complete loss in plasticity. This was in agreement with the increased TS in the samples added with 100% lipid (100% glycerol substitution). In absence of glycerol, no differences in EAB were observed among all films with 100% lipid regardless of lipid types. In general, low-molecular-weight plasticizers are added to protein film in order to improve film flexibility by reducing protein-protein interaction (Krochta, 1997).

Table 9 Tensile strength (TS), elongation at break (EAB), water vapor permeability (WVP) and thickness of bigeye snapper surimi film as affected by types and amounts of lipids.

Films	Lipid conc. (%)	TS[#] (MPa)	EAB[#] (%)	WVP[##] (x 10^{-10} $gm^{-1}s^{-1}Pa^{-1}$)	Thickness[#] (mm)
Control	0	3.55 ± 0.77^{b}*	73.73 ± 3.48^{cd}	1.15 ± 0.08^{a}	0.030 ± 0.001^{d}
Palm oil	25	0.72 ± 0.05^{fg}	77.94 ± 1.39^{c}	0.95 ± 0.07^{b}	0.031 ± 0.001^{cd}
	50	1.02 ± 0.25^{ef}	83.13 ± 4.96^{b}	0.93 ± 0.12^{bc}	0.032 ± 0.002^{bcd}
	75	1.34 ± 0.19^{e}	102.50 ± 10.89^{a}	0.80 ± 0.12^{d}	0.032 ± 0.001^{bcd}
	100	3.39 ± 0.66^{b}	2.47 ± 0.44^{g}	0.78 ± 0.06^{d}	0.032 ± 0.001^{bc}
Butter	25	0.51 ± 0.03^{g}	73.23 ± 1.54^{cd}	0.93 ± 0.15^{bc}	0.031 ± 0.001^{cd}
	50	0.63 ± 0.09^{fg}	66.75 ± 3.02^{e}	0.93 ± 0.07^{bc}	0.032 ± 0.002^{bcd}
	75	1.90 ± 0.31^{d}	60.07 ± 7.96^{f}	0.81 ± 0.06^{cd}	0.031 ± 0.001^{cd}
	100	2.54 ± 0.56^{c}	1.26 ± 0.78^{g}	0.78 ± 0.08^{d}	0.033 ± 0.001^{ab}
Shortening	25	0.99 ± 0.14^{ef}	71.46 ± 3.17^{d}	1.10 ± 0.08^{a}	0.032 ± 0.001^{bcd}
	50	0.77 ± 0.13^{fg}	63.38 ± 3.44^{ef}	1.08 ± 0.08^{a}	0.032 ± 0.001^{bcd}
	75	1.79 ± 0.36^{d}	63.04 ± 4.30^{ef}	0.88 ± 0.22^{bcd}	0.033 ± 0.001^{ab}
	100	4.10 ± 0.75^{a}	1.92 ± 1.20^{g}	0.87 ± 0.09^{bcd}	0.034 ± 0.001^{a}

[#]Mean±SD from eight determinations.

[##]Mean±SD from four determinations.

*The different superscripts in the same column indicate the significant differences ($P<0.05$).

4.2 Water vapor barrier property of bigeye snapper surimi film

Surimi films have typically high WVP due to hydrophilic nature of protein. Transmission of water vapor through surimi film is also facilitated by the presence of glycerol, a hydrophilic plasticizer which favorably absorbs the water or moisture (Cuq et al., 1995). Additionally, cryoprotectants including sucrose and sorbitol in surimi also provided the polar groups in the film. Those polar groups provide for hydrogen bonding with water (Prodpran and Benjakul, 2005). As a result, the surimi film could absorb the water from the surrounding air or from the food product. To prevent the moisture migration into the surimi film, hydrophobic substance such as lipids are commonly used (Morillon et al., 2002).

Table 9 shows WVP of bigeye snapper surimi film as affected by different types and amounts of lipids added. In general, WVP of film added with palm oil or butter decreased when the added lipid increased ($P<0.05$). Among all samples, the control containing 100% glycerol (without lipid addition) possessed the greatest WVP. From the result, the increasing amount of lipids increased the barrier efficiency against moisture transfer of film by increasing the hydrophobic substances, while lowering the hydrophilic glycerol amount (Martin-Polo et al, 1992; Morillon et al., 2002; Yang and Paulson, 2000; Bertan et al., 2005). Yang and Paulson (2000) reported that both beeswax and the blend of stearic and palmitic acids effectively reduced WVP of gellan film. The solid or liquid state of lipids strongly influenced the barrier efficiency of the film. It has been reported that the increase of the solid fat content allowed for the improvement of the barrier efficiency (Martin-Polo et al., 1992; Yang and Paulson, 2000; Bertan et al., 2005). This is because CH_2 groups of

liquid aliphatic chains have a greater volume than when they are crystallized (Morillon et al., 2002).

However, WVP of films added with shortening at 25 and 50% glycerol substitution was not different from that of the control ($P>0.05$). Though the shortening was incorporated as the hydrophobic substance, it might cause the formation of fat crystal and the micro-crack or the disconnection of interstitial zone could occur. This structural defects within the film might result in some leakage of film. Sapru and Labuza (1994) reported that the moisture migration was favored when the formation of large crystals of stearic acid was formed, allowing interstitial zones free of lipid. Therefore, the difference in WVP between film added with two solid lipids, butter and shortening, was possibly owing to the differences in lipid nature, particularly their crystallinity as well as fatty acid composition. Water vapor permeability of film was highly dependent on fatty acid chain length and degree of saturation (Morillon et al., 2002; Pommet et al., 2003).

4.3 Color and transparency of surimi film

Bigeye snapper surimi film became more lighter as evidenced by the increase in L*-value when the amount of lipid incorporated in the film increased ($P <0.05$) (Table 10). The decrease in a*-value was noticeable as the lipids was incorporated into the films ($P<0.05$). However, no differences were observed among all samples added with varying types and amounts of lipids ($P>0.05$). For b*-value, films added with palm oil or shortening had the lowered value, compared with that of the control. On the other hand, b*-value of film added with butter increased as the

amount of butter added increased ($P<0.05$). This indicated the increasing yellowness of film, possibly due to the greater amount of pigments naturally found or added in the butter.

Lower transparency of surimi film as indicated by the greater values was found when the greater amount of lipids was incorporated ($P<0.05$) (Table 10). At the same level of lipid used, films with palm oil addition showed less opacity or more transparency than those added with butter or shortening. Among all samples, film added with shortening was the most opaque. Increasing opacity of the protein film by addition of hydrophobic substances has been reported (Bertan et al., 2005; Pommet et al., 2003; Yang and Paulson, 2000; Gontard et al., 1994). The increase in opacity was possibly caused by the light scattering from the lipid droplets distributed throughout the protein network. The surface of solid lipid droplet most likely exhibited more light scattering effect than that of liquid lipid. The difference in crystallinity and surface of both solid lipids might contribute to the different light scattering of resulting film. Yang and Paulson (2000) reported that the differences in opacity of film were determined by the optical properties of lipids incorporated. Solid fat was also reported to increase the opacity of polysaccharide-based film (Gallo et al., 2000).

Table10 Color parameters and transparency of bigeye snapper surimi film as affected by types and amounts of lipids.

Films	Lipid conc. (%)	L*[#]	a*[#]	b*[#]	Transparency[#]
Control	0	90.18±0.13^{g}*	-1.33±0.06^{a}	3.39±0.05^{c}	5.83±0.02^{l}
Palm oil	25	90.65±0.01^{f}	-1.58±0.04^{b}	2.80±0.03^{fg}	9.86±0.00^{k}
	50	91.43±0.02^{e}	-1.64±0.04^{bcd}	2.65±0.05^{h}	11.72±0.15^{j}
	75	92.06±0.05^{c}	-1.60±0.05^{bc}	2.81±0.01^{fg}	19.35±0.01^{f}
	100	92.50±0.02^{a}	-1.70±0.03^{cd}	2.76±0.04^{g}	21.00±0.01^{d}
Butter	25	91.46±0.02^{e}	-1.60±0.02^{bc}	2.76±0.03^{g}	13.93±0.01^{i}
	50	91.64±0.02^{d}	-1.66±0.04^{bcd}	2.97±0.03^{e}	16.31±0.05^{h}
	75	92.02±0.02^{c}	-1.65±0.10^{bcd}	4.34±0.05^{b}	22.99±0.05^{c}
	100	92.23±0.05^{b}	-1.91±0.07^{c}	4.83±0.04^{a}	30.97±0.17^{b}
Shortening	25	90.66±0.03^{f}	-1.71±0.06^{cd}	2.62±0.03^{h}	17.11±0.06^{g}
	50	91.43±0.02^{e}	-1.76±0.08^{d}	3.37±0.05^{c}	20.72±0.02^{e}
	75	91.64±0.06^{d}	-1.67±0.08^{bcd}	3.14±0.05^{d}	22.87±0.06^{c}
	100	92.30±0.15^{b}	-1.66±0.10^{bcd}	2.85±0.03^{f}	36.07±0.04^{a}

[#]Mean±SD from three determinations.

*The different superscripts in the same column indicate the significant differences ($P<0.05$).

4.4 Film solubility and protein solubility of bigeye snapper surimi film

Table 11 shows water solubility of the bigeye snapper surimi films with and without lipid modification. For the control, solubility of 67.69% was noted. The loss in film solubility of the control was owing to the aggregation of proteins in the film network. Film solubility markedly decreased when the lipids were incorporated. The loss in solubility was pronounced when the greater levels of lipids were incorporated. The solubility decreased to a greater degree as the higher lipid amount was added. Water solubility is an indicative of the film hydrophilicity.

Addition of lipid thus increased hydrophobicity of the surimi film. Similar results were reported by Kim and Ustunol (2001) on lipid-whey protein emulsion films. From the results, the higher lipid content used was concomitant with the lower glycerol content in the film. As a consequence, the glycerol dissolved in extracting water was lowered when the percent lipid substitution was increased. Also, the lowered preventive effect of glycerol towards the protein aggregation was presumed. As previously discussed, the hydrophobic lipids were rarely dispersed throughout the very polar protein-rich phase. Without the sufficient glycerol, a hydrophilic plasticizer, the more aggregation took place, leading to a great loss in film solubility. At the same level of lipid used, film added with palm oil showed the lowest solubility, and the greater solubility's were observed for the films with butter and shortening additions.

Similar results were observed with protein solubility (Table 11). Surimi film was formed by the cross-links stabilized by various bonding including hydrogen bond, hydrophobic interaction as well as disulfide bond (Shiku et al., 2003). The cross-linked proteins in the surimi film generally tended to lose their solubility. However, the loss in protein solubility was more pronounced as the lipid used increased, suggesting the greater aggregation of proteins in the film. This was in accordance with the increase in TS and the decrease in EAB of the film added with a greater amount of lipids (Table 9).

Table 11 Film solubility and protein solubility of bigeye snapper surimi film as affected by types and amounts of lipids.

Films	Lipid conc. (%)	Film solubility[#] (%)	Protein solubility[#] (%)
Control	0	67.69±0.21[a]*	26.78±2.64[a]
Palm oil	25	34.65±1.21[ef]	17.61±1.16[bc]
	50	32.13±0.38[g]	16.83±1.26[bc]
	75	30.36±0.98[h]	14.31±0.22[cd]
	100	27.04±0.45[i]	10.40±0.59[d]
Butter	25	36.33±1.03[d]	20.79±4.90[b]
	50	35.60±0.45[de]	16.32±2.99[bc]
	75	34.30±0.38[ef]	14.27±2.98[cd]
	100	33.97±0.64[f]	13.94±3.59[cd]
Shortening	25	42.88±0.95[b]	17.11±3.62[bc]
	50	42.66±0.57[b]	17.01±2.45[bc]
	75	40.99±1.13[c]	14.99±1.26[cd]
	100	40.96±0.58[c]	14.72±1.51[cd]

[#]Mean±SD from three determinations.

*The different superscripts in the same column indicate the significant differences ($P<0.05$).

4.5 Protein pattern of bigeye snapper surimi film

SDS-PAGE protein patterns of surimi films added with different types and amounts of lipids are depicted in Figure 8. It was found that the control film contained both myosin heavy chain (MHC) and actin as the major constituents. In general, MHC band intensity increased when the levels of lipids incorporated increased. The MHC band intensity of sample added with lipid at 100% glycerol substitution was similar to that found in the surimi (data not shown). The result

suggested that non-disulfide covalent bond was formed in the control film (without lipid addition) to some extent. Reducing sugar in the film forming solution might undergo Maillard reaction with the amino groups of protein, leading to the covalent cross-linking of proteins. The reaction might be enhanced during the drying process of films. Chinabhark et al. (2005) reported that the reducing sugar was produced in acidic film forming solution of frozen surimi from bigeye snapper. Polymerization of protein was induced by Maillard glycation (Chevalier et al., 2002)

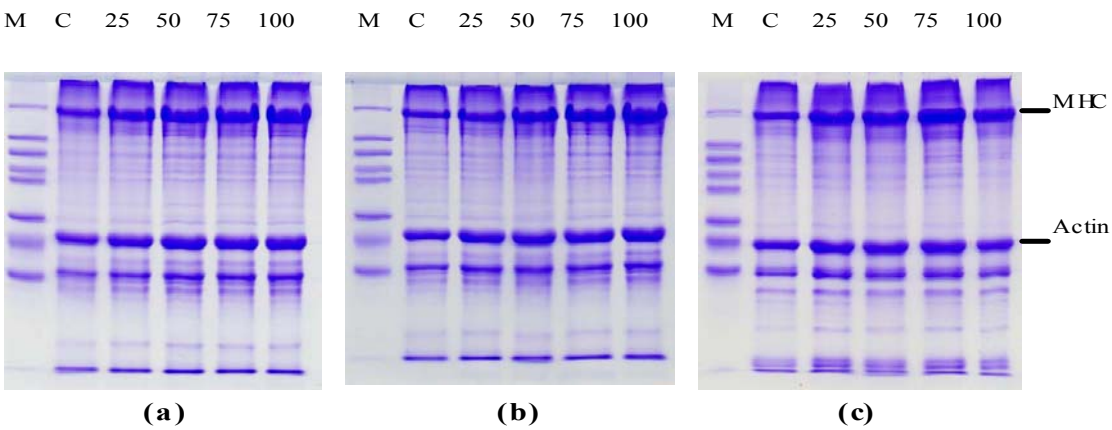
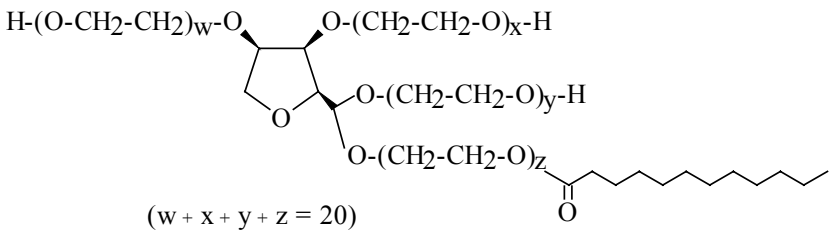


Figure 8 Protein patterns of bigeye snapper surimi films incorporated with palm oil (a), butter (b) and shortening (c). M: high-molecular-weight protein marker; C: control film (without lipid). Numbers denote lipid levels as % glycerol substitution

As the lipid was added to a greater extent, this bonding was much reduced as evidenced by the greater MHC band intensity retained. Tween-20 might play a role in impeding the non-disulfide covalent bond formation in a concentration

dependent manner. Tween-20, a nonionic surfactant [I], was added proportionally to the amount of lipids used.



Tween-20 [I]

Tween-20 localized at the interface by exposing the hydrophobic portion to lipid phase. The hydrophilic portion was imposed in the protein-rich phase. With increasing Tween-20 at the interface, the reducing sugars might migrate to hydrophilic shield of interface surrounded by Tween-20. The diffusion of sugar through colloidal dispersions has been reported in literatures (Basaran and McClements, 1999; Matsumoto et al., 2000). As a consequence, the lower amount of reducing sugar was retained in the protein-rich phase and Maillard reaction was decreased. As a result, the non-disulfide cross-links via Maillard glycation was reduced. Although the degradation of MHC in edible films based on sardine myofibrillar proteins, especially in the acidic pH ranges, due to the cathepsins was reported (Cuq et al., 1995), the negligible degradation was noted in the acidic film forming solution of bigeye snapper surimi (Chinabhark et al., 2005). Furthermore, Tween-20 showed no inhibitory effect towards proteolysis (data not shown). From the

result, similar protein patterns were obtained among films added with different lipids. This confirmed that lipid types had no pronounced effect on protein pattern of the surimi protein/lipid composite film.

4.6 Surface characteristics

The SEM micrographs of surimi film incorporated with palm oil at different levels as the substitute of glycerol are shown in Figure 9. The control film (without lipids) had the smooth and continuous surface without grainy and porous structure. This indicated that the film with ordered network was formed without the air bubbles. With addition of palm oil, the surface of surimi film had the irregular surface with the distribution of oil droplets. The oil droplets were more intense on the surface as the amount of oil incorporated increased. The continuously spread oil droplets on the film surface might be associated with the reduced WVP of film with a greater amount of oil (Table 9).

When the surface characteristics of films added with different lipids, palm oil, butter and shortening, at 75% glycerol substitution were compared (Figure 10), different surface was observed. The greatest irregularity was noticeable with the film containing shortening. The large and irregular shaped-droplets were found throughout the film. For the butter containing film, smaller droplet of butter was found to distribute in the film, compared with that obtained in shortening containing film. The differences in surface among all films tested might be caused by the different crystallinity of lipids used. This might result in the varying distribution and solidity of lipids in films. The surface characteristics of gellan film were determined

by varying lipid types and the differences in surface structure might contribute to some extent to the differences in WVP of the films (Yang and Paulson, 2000).

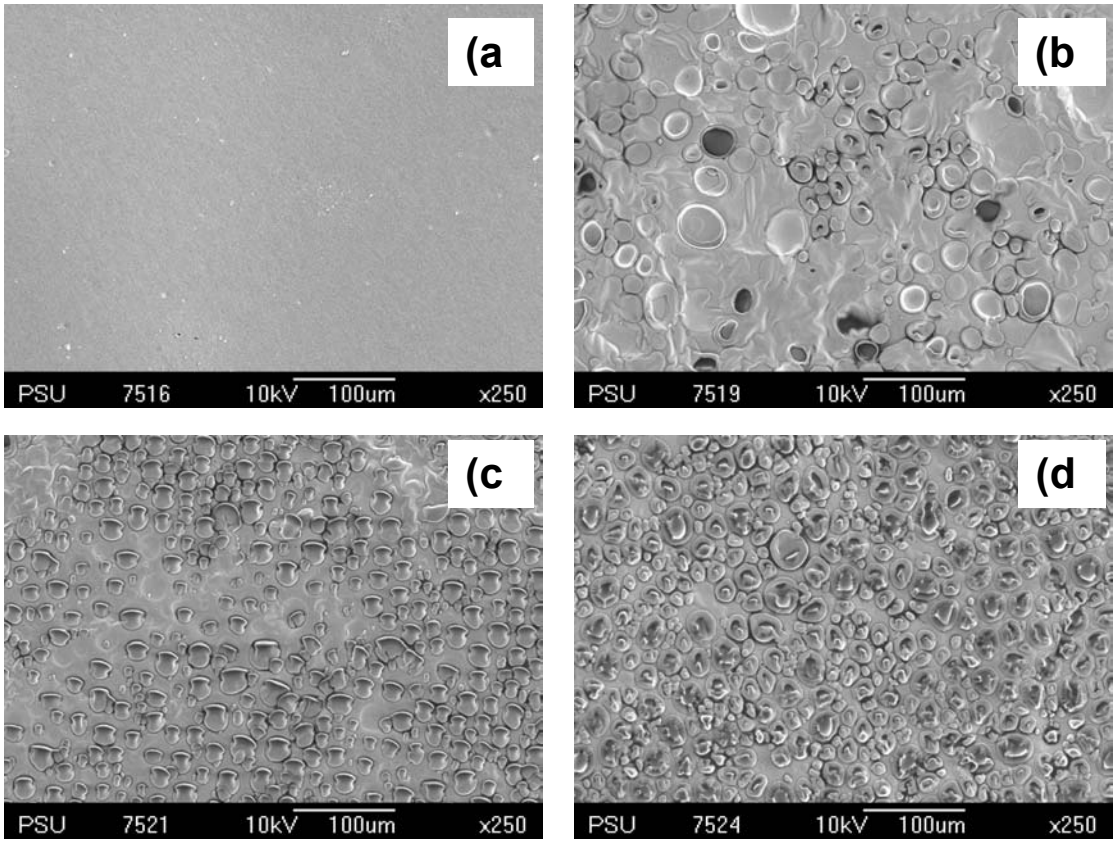


Figure 9 SEM micrographs of bigeye snapper surimi films incorporated without and with palm oils at different levels. Control film (without lipid) (a) and films incorporated with palm oil at 25% (b), 75% (c) and 100% (d) glycerol substitution.

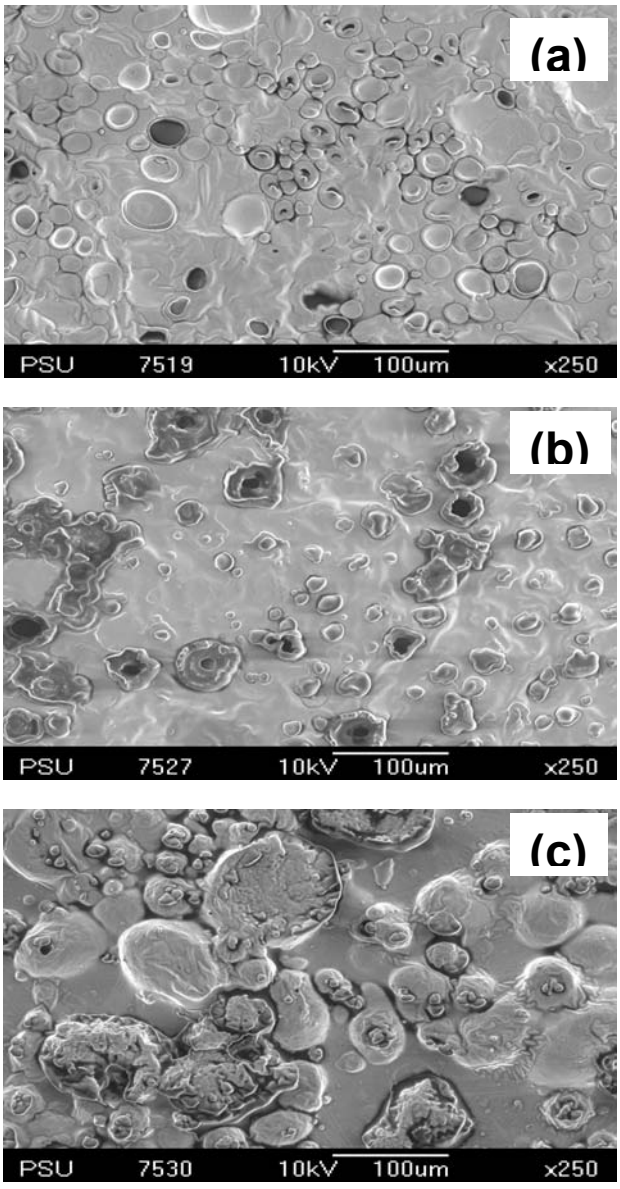


Figure 10 SEM micrographs of bigeye snapper surimi films incorporated with palm oil (a), butter (b) and shortening (c) at 75% glycerol substitution.

From the result, bigeye snapper surimi films added with palm oil at 75% glycerol substitution had the highest mechanical property and lowest WVP, compared with other samples. Therefore, bigeye snapper surimi films added with palm oil at 75% glycerol substitution was chosen and used for further study.

4.7 Moisture sorption isotherms

Moisture sorption isotherms of bigeye snapper surimi film added with palm oil at 75% glycerol substitution determined at 4ºC and room temperature (28-30º C) are depicted in Figure 11. Moisture sorption isotherms presented a sigmoid shape in common with those of most foods. At low water activities (0.15-0.45), moisture content of the films determined at both 4ºC and room temperature increased slowly. Moisture content of the films increased rapidly at A_w greater than 0.75 ($P>0.05$). From the result, keeping temperature did not show the marked influence on the moisture sorption isotherm of surimi film added with palm oil. Palm oil incorporated into the surimi film might prevent or retard the absorption of water into the film.

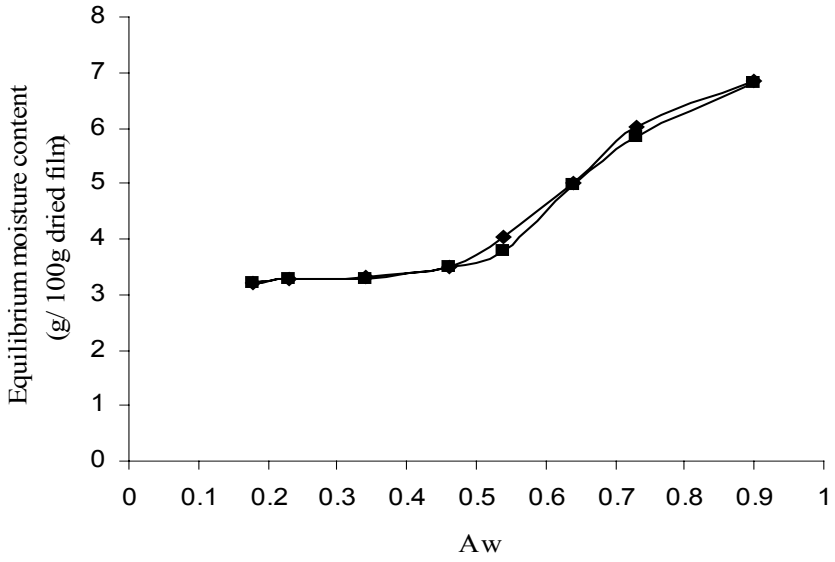


Figure 11 Moisture sorption isotherms of bigeye snapper surimi film added with palm oil at 75% glycerol substitution at 4ºC (■and room temperature (.)▲

5. Effect of crosslinker on the properties of bigeye snapper surimi film incorporated with palm oil

5.1 The use of aldehydes

5.1.1 Mechanical properties of bigeye snapper surimi film

The properties of films incorporated with palm oil at 75% glycerol substitution in the presence of different types and levels of aldehydes are shown in Table 12. Surimi film had a slight increase in thickness as the levels of aldehydes added increased ($P<0.05$) (Table 12). Addition of aldehydes, cross-linking agents, to the film-forming solution resulted in the increase in TS of films obtained ($P<0.05$). The formation of more resistant films suggests the occurrence of new covalent bonds

between proteins in surimi film via chemical reaction between aldehydes and amino acid side chain reactive groups. Increasing TS of the protein film by incorporation of aldehyde agents has been reported (Ghorpade et al., 1995; Rhim et al., 2000; Parris and Coffin, 1997). At the same concentration of aldehyde used, TS values of films incorporated with formaldehyde was greater than those of films added with glutaraldehyde and glyoxal. The result was in agreement with Hernández-Muñoz et al. (2004) who reported that formaldehyde was more efficient than glutaraldehyde and glyoxal in cross-linking glutenin-rich films. Marquié et al. (1995) showed that formaldehyde was more efficient than glutaraldehyde and gossypol for cross-linking cottonseed protein films. This suggests that formaldehyde is a low molecular weight molecule and could easily migrate between the protein chains and establish new covalent bonds with Lys, Cys and His groups of the proteins in whey protein films (Gallieta et al., 1998). From the result, the increased TS was observed with increasing aldehyde levels, except for films added with glutaraldehyde, which no changes were found with increasing concentrations used (0.5-5 mM).

Table 12 shows EAB of films added with different aldehydes at varying levels. EAB of films treated with all aldehydes showed the lower EAB than that of the control films ($P<0.05$). EAB decreased gradually when formaldehyde, glutaraldehyde and glyoxal were incorporated up to 5 mM. As shown in Table 12, the lowest EAB values were observed with film added with formaldehyde. At the same level of aldehyde used, glyoxal incorporated films showed the highest EAB. EAB decrease obtained after cross-liking treatment of protein films was in agreement with the development of a more rigid structure. Similar results were reported for gelatin films cross-linked with glutaraldehyde (Bigi, 1998; 2001), wheat gluten films treated

with formaldehyde vapours (Micard et al., 2000) and glutenin-rich films cross-linked with formaldehyde, glutaraldehyde and glyoxal (Hernández-Muñoz et al., 2004). Micard et al. (2000) observed that wheat gluten films treated with formaldehyde showed a decrease in EAB, when compared with the untreated control films.

5.1.2 Water vapor barrier property of bigeye snapper surimi film

WVP of bigeye snapper surimi film incorporated with palm oil as affected by different types and amounts of aldehydes added is shown in Table 12. Cross-linked films showed lower WVP than the control film. The decrease in WVP was found in film treated with formaldehyde as the concentration used increased ($P < 0.05$); however no changes in WVP was found in the surimi film treated with glutaraldehyde and glyoxal. From the result, the increasing reticulation of the film network with low molecular weight aldehydes could decrease the free volume of the polymeric matrix and increase the tortuosity of the pathway of the water molecules through the network, thus decreasing diffusion rate of water molecules through the films. Liu et al. (2004) reported that the ability of formaldehyde and glutaraldehyde to promote intermolecular and intramolecular cross-linking of peanut protein film was found to increase the barrier properties of the films. Micard et al. (2000) observed that gluten based films treated with formaldehyde had a slight reduction in WVP.

Normally, an increase in crystallinity, orientation, molecular mass or degree of cross-linking results in a decrease in permeability (Miller and Krochta, 1997). From the result, it was suggested that aldehydes increased the barrier efficiency against moisture transfer of film by the structural arrangement obtained

after the introduction of the cross-linkages and also by the degree of cross-linking formed, which could favor the decrease of permeant diffusion in the modified polymeric matrix (Bigi et al., 2001; de Carvalho and Grosso, 2004).

Table 12 Tensile strength (TS), elongation at break (EAB), water vapor permeability (WVP) and thickness of bigeye snapper surimi film incorporated with palm oil as affected by types and amounts of aldehydes.

Films .	TS[#] (MPa)	EAB[#] (%)	WVP[##] (x 10^{-10}gm^{-1}s^{-1}Pa^{-1})	Thickness[#] (mm)
Control	1.41±0.16^{f}*	99.97±2.83^{a}	0.80±0.07^{a}	0.032±0.001^{e}
Formaldehyde (mM)				
0.5	3.23±0.09^{b}	53.58±1.27^{g}	0.65±0.10^{bc}	0.042±0.001^{bcd}
1	3.31±0.12^{b}	48.33±2.29^{h}	0.62±0.08^{bc}	0.043±0.001^{bc}
5	3.72±0.25^{a}	47.91±2.21^{h}	0.44±0.15^{d}	0.048±0.001^{a}
Glutaraldehyde (mM)				
0.5	2.96±0.11^{c}	70.60±1.86^{d}	0.72±0.08^{abc}	0.041±0.003^{cd}
1	3.04±0.08^{c}	68.00±1.75^{e}	0.71±0.10^{abc}	0.042±0.001^{bcd}
5	3.08±0.11^{c}	59.86±4.07^{f}	0.61±0.13^{c}	0.048±0.001^{a}
Glyoxal (mM)				
0.5	2.32±0.06^{e}	93.40±2.06^{b}	0.77±0.08^{ab}	0.040±0.001^{d}
1	2.42±0.06^{e}	93.06±2.08^{b}	0.74±0.13^{abc}	0.041±0.001^{bcd}
5	2.65±0.09^{d}	56.48±1.08^{c}	0.63±0.08^{bc}	0.043±0.001^{b}

[#]Mean±SD from eight determinations.

[##]Mean±SD from four determinations.

*The different superscripts in the same column indicate the significant differences ($P < 0.05$).

5.1.3 Color and transparency of bigeye snapper surimi film

Bigeye snapper surimi film incorporated with palm oil became more darker as evidenced by the decrease in L*-values when the amount of formaldehyde, glutaraldehyde and glyoxal added into the film increased ($P<0.05$). The increase in b*-value was noticeable as all aldehydes used increased ($P<0.05$). Films incorporated with formaldehyde had a slightly lighter and less yellowish brown than films incorporated with glutaraldehyde and glyoxal. The increasing yellowness of film added with aldehydes was possibly associated with protein-aldehydes interactions via the Maillard reaction (Damodaran, 1996).

Surimi film incorporated with various aldehydes at different levels had different transparency (Table 13). At the same level of aldehydes used, films added with formaldehyde were more transparent than those incorporated with glutaraldehyde and glyoxal. Films turned to be more opaque with increasing aldehydes added. Increasing opacity of the protein film by incorporation of aldehydes has been reported (Bertan et al., 2005; Pommet et al., 2003; Yang and Paulson, 2000; Gontard et al., 1994). The increase in opacity was possibly caused by the orientation from the polymeric matrix throughout the protein network.

Table 13 Color parameters and transparency of bigeye snapper surimi film incorporated

with palm oil as affected by types and amounts of aldehydes.

Films .	L*[#]	a*[#]	b*[#]	Transparency[#]
Control	92.05±0.04[b*]	-1.75±0.08[d]	2.78±0.08[d]	19.33±0.01[g]
Formaldehyde (mM)				
0.5	91.05±0.01[g]	-1.41±0.34[ab]	1.93±0.03[e]	19.41±0.02[f]
1	91.17±0.01[f]	-1.34±0.05[a]	2.10±0.02[e]	19.50±0.03[e]
5	91.25±0.03[e]	-1.64±0.03[cd]	2.66±0.10[d]	19.57±0.02[d]
Glutaraldehyde (mM)				
0.5	91.47±0.01[d]	-1.61±0.02[bcd]	3.14±0.03[c]	19.47±0.01[e]
1	90.88±0.01[h]	-1.52±0.03[abc]	5.39±0.03[b]	19.79±0.01[c]
5	89.82±0.01[i]	-1.31±0.08[a]	6.11±0.05[a]	19.87±0.04[b]
Glyoxal (mM)				
0.5	92.41±0.01[a]	-1.82±0.02[d]	2.05±0.04[e]	19.78±0.01[c]
1	91.65±0.01[c]	-1.79±0.59[d]	3.06±0.03[c]	19.81±0.02[c]
5	90.88±0.01[h]	-1.65±0.07[cd]	3.07±0.05[c]	20.55±0.01[a]

[#]Mean±SD from three determinations.

*The different superscripts in the same column indicate the significant differences ($P<0.05$).

5.1.4 Film solubility and protein solubility of bigeye snapper surimi film

Table 14 shows water solubility and protein solubility of bigeye snapper surimi film added with palm oil and different aldehydes at various concentrations in comparison with the control film. For the control, solubility of 29.84% was noted. It is well known that myofibrillar proteins present extremely low solubility in water especially when the protein molecules underwent cross-linking

during film formation. The loss in film solubility was observed with increasing amount of aldehydes added (Table 14). The loss in solubility was more pronounced when the greater levels of aldehydes were incorporated. At the same amount of aldehydes used, film added with formaldehyde had the lower solubility than those with glutaraldehyde and glyoxal added. Glyoxal cross-linked films showed the highest film solubility. The result suggested the different efficacy in cross-linking of protein among different aldehyde cross-linkers. The result was in agreement with Micard et al. (2000) who found the decrease in water solubility of gluten-based films as a result of formaldehyde treatment. Defatted cotton protein films, 100% soluble in water, decreased in water solubility to approximately 30% after cross-linking with formaldehyde at 2.86 mM (Marquié et al., 1995). Galietta et al. (1998) observed a decrease in water solubility from 43 to 30% in milk whey protein based films treated with formaldehyde, as compared to unmodified films.

Similar results were observed with protein solubility (Table 14). However, lower value of protein solubility was noticeable, compared with that of film solubility. Glycerol used as plasticizer might contribute to the higher value for film solubility since it was water soluble. Gelatin based films modified with glyoxal and formaldehyde contained the cross-links stabilized by covalent bonds (de Carvalho and Grosso, 2004). The cross-linked proteins in the protein film generally tended to lose their solubility. However, the loss in protein solubility was more pronounced as the aldehydes used increased, suggesting the greater aggregation of proteins in the film. Aldehydes treatment possibly led to a reduction in the low molecular weight fractions via cross-linking, thus decreasing the solubility of protein in the films. Proteins in film treated with formaldehyde totally lost their solubility. Glutaraldehyde and glyoxal

also resulted in the decrease in protein solubility at different degrees. The result was in accordance with that of film solubility. The increases in TS and the decrease in EAB of the film incorporated with aldehydes were in agreement with the losses in film or protein solubility (Table 12).

Table 14 Film solubility and protein solubility of bigeye snapper surimi film incorporated with palm oil as affected by types and amounts of aldehydes.

Films	Film solubility[#] (%)	Protein solubility[#] (%)
Control	29.84±0.77[a*]	14.15±1.64[a]
Formaldehyde (mM)		
0.5	8.86±0.51[g]	ND
1	6.18±0.49[h]	ND
5	5.25±0.18[h]	ND
Glutaraldehyde (mM)		
0.5	16.09±1.72[d]	4.33±1.12[c]
1	14.33±0.34[e]	2.10±0.09[d]
5	12.44±0.61[f]	0.68±0.01[d]
Glyoxal (mM)		
0.5	21.30±0.87[b]	9.24±0.43[b]
1	18.90±1.24[c]	7.54±2.12[b]
5	18.69±0.60[c]	7.08±1.57[ab]

[#]Mean±SD from three determinations.

*The different superscripts in the same column indicate the significant differences ($P<0.05$).

ND: non-detectable.

5.1.5 Protein patterns of bigeye snapper surimi film

SDS-PAGE protein patterns of surimi films added with palm oil in the presence of different types and amounts of aldehydes are depicted in Figure 12. In general, MHC band intensity decreased when the levels of aldehydes incorporated increased. However, intensity of actin band also decreased when a higher amount of aldehyde was used. Generally, MHC of films was more susceptible to cross-linking by formaldehyde, glutaraldehyde and glyoxal in the descending order. However, actin was not polymerized in the presence of glyoxal at levels up to 5 mM. This indicated the different efficacy in cross-linking of protein by different aldehydes. The result suggested that covalent bond was formed in the film to some extent. Aldehydes are able to form covalent inter- and/or intra-molecular links between protein chains (Gennadios and Weller, 1992; Marquié et al., 1995). The formation of new covalent bonds might be enhanced during the film-forming process.

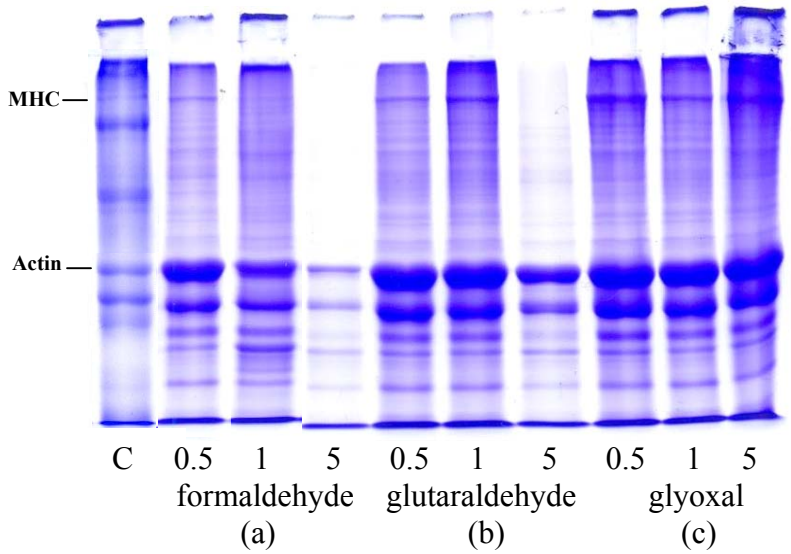


Figure 12 Protein patterns of bigeye snapper surimi films containing palm oil and incorporated with formaldehyde (a), glutaraldehyde (b) and glyoxal (c). C: control film (without aldehyde). Numbers denote aldehyde levels (mM).

5.2 The use of microbial TGase

5.2.1 Mechanical properties of bigeye snapper surimi film

Films containing palm oil and added with different amounts of MTGase were slightly different in thickness (Table 15). TS of bigeye snapper surimi film added with MTGase at various levels is shown in Table 15. In general, the addition of MTGase (5, 10, 20, 40 and 80 units/ g protein) to surimi films caused the decrease in TS. The results obtained were in contrast with other works reported elsewhere. Yildrim and Hettiarachchy (1998) observed an increase in TS of MTGase modified milk whey and globulin 11S based films. Babin and Dickinson (2001) observed that gelatin films treated with transglutaminase could present both positive

and negative effects on the strength of gelatin Types A and B, depending on the order in which the cross-linkages were formed, before or after formation of junction zones induced by the cooling of the solution to temperatures below 35ºC. Conversely, TS of surimi films generally decreased as the MTGase levels used increased up to 80 units/ g proteins ($P<0.05$). Strength reduction of edible films with MTGaes incorporation has been reported (de Carvalho and Grosso, 2004). Oil added in the film network might reduce the efficacy of MTGase in crosslinking proteins intermolecularly via increasing the hydrophobicity of film forming solution. As a consequence, the hydrophilic enzyme could not induce the cross-linking of protein effectively.

EAB of surimi films increased with increasing MTGase levels (5, 10, 20, 40 and 80 units/ g protein) (Table 15). This result suggested that structured networks of myofibrillar proteins could also be formed in the presence of transglutaminase, which catalyze formation of intermolecular isopeptidic bonds (Kim et al., 1993). The probability of forming intermolecular bonds mainly depends on protein conformation. Myofibrillar protein, fibrous proteins generally can form films with good mechanical properties (Cuq et al., 1998). From the result, it was postulated that MTGase might induce the connection of protein molecules, in which the longer chains were formed. However, the cross-linking of adjacent proteins could be limited. As a consequence, the strengthened/ dense network could not be formed but the looser network would be obtained instead, which resulted in increasing film extensibility. The mechanism of cross-linking of surimi protein by aldehydes and MTGase might be different, leading to the different mechanical property of resulting films. Therefore, addition of MTGase could improve the extensibility of surimi film.

5.2.2 Water vapor barrier property of bigeye snapper surimi film

WVP of bigeye snapper surimi film containing palm oil as affected by different amounts of MTGase added is shown in Table 15. No changes in WVP of film added with MTGase were observed when the amount of added MTGase increased ($P>0.05$). The result was in agreement with Mahmoud and Savello (1992) who reported that cross-linking of β-Lg, α-La and whey protein isolate film by transglutaminase in the presence of Ca^{+2} had no effect on water vapor transmission rate and moisture content of films. However, WVP of transglutaminase-cross-linked whey protein isolate films was lower than that of control films (Yildirim and Hettarachchy, 1998). From the result, the cross-linking of proteins induced by MTGase could not orient the protein molecules in the fashion which reduces the migration of water into the film.

Table 15 Tensile strength (TS), elongation at break (EAB), water vapor permeability (WVP) and thickness of bigeye snapper surimi film incorporated with palm oil as affected by MTGase levels.

MTGase (units/g protein)	TS[#] (MPa)	EAB[#] (%)	WVP[##] (x 10^{-10} $gm^{-1}s^{-1}Pa^{-1}$)	Thickness[#] (mm)
0 (control)	1.48±0.21[a*]	4.99±0.70[c]	0.78±0.05[a]	0.045±0.001[d]
5	1.71±0.23[a]	5.71±0.75[c]	0.78±0.03[a]	0.045±0.001[d]
10	1.44±0.62[a]	19.66±5.76[b]	0.75±0.11[a]	0.046±0.001[c]
20	1.06±0.08[b]	24.07±5.46[a]	0.73±0.10[a]	0.046±0.001[c]
40	0.97±0.07[b]	26.55±4.00[a]	0.72±0.05[a]	0.047±0.001[b]
80	0.94±0.23[b]	28.10±2.67[a]	0.71±0.09[a]	0.048±0.001[a]

[#]Mean±SD from eight determinations.
[##]Mean±SD from four determinations.

*The different superscripts in the same column indicate the significant differences ($P<0.05$).

5.2.3 Color and transparency of bigeye snapper surimi film

L*, a* and b*-values of bigeye snapper surimi film containing palm oil and added with different amounts of MTGase are shown in Table 16. Bigeye snapper surimi film became more lighter than the control films as evidenced by the slight increase in L*-values when the amount of MTGase added increased ($P<0.05$). The increase in a*-value was noticeable as MTGases amount increased ($P<0.05$). For b*-value, films added with a greater amount of MTGase had the higher values. This indicated the increasing yellowness of film, possibly because the greater amount of MTGase could induce the conformational changes in the way which made the amino group accessible to Maillard reaction (Babiker et al, 1998).

The transparency of surimi film added with MTGase was lower than that of control films as shown in Table 16. The transparency values increased with increasing amount of MTGase used ($P<0.05$) (Table 16) indicating the decrease in transparency of films. An increase in MTGase levels resulted in more opacity as indicated by the increase in transparency. The increase in opacity was possibly caused by protein polymerization induced by MTGase (Faergemand et al., 1999).

Table 16 Color parameters and transparency of bigeye snapper surimi film incorporated with palm oil as affected by MTGase levels.

MTGase (units/g protein)	L*[#]	a*[#]	b*[#]	Transparency[#]
0 (control)	90.15±0.01[d]*	-1.92±0.06[d]	2.89±0.04[c]	27.86±0.97[c]
5	90.27±0.02[c]	-1.65±0.01[c]	2.97±0.03[c]	32.63±0.21[b]
10	90.31±0.01[b]	-1.59±0.11[bc]	3.02±0.05[c]	33.01±0.10[b]
20	90.32±0.02[b]	-1.57±0.06[bc]	3.05±0.06[c]	33.24±0.08[b]
40	90.70±0.01[a]	-1.50±0.07[ab]	3.28±0.19[b]	34.81±0.02[a]
80	90.64±0.01[a]	-1.44±0.05[a]	3.95±0.05[a]	35.49±0.05[a]

[#]Mean±SD from three determinations.
*The different superscripts in the same column indicate the significant differences ($P<0.05$).

5.2.4 Film solubility and protein solubility of bigeye snapper surimi film

Table 17 shows film solubility and protein solubility of the bigeye snapper surimi films containing palm oil with and without MTGase modification. For the control film, solubility of 25.34% was obtained. Solubility of MTGase cross-linked films were decreased, compared with that of the control films ($P<0.05$). The loss in film solubility was owing to the aggregation of proteins in the film network.

The loss in solubility was pronounced when the greater levels of MTGase were added. Only small fraction of MTGase cross-linked protein samples remained in the monomeric form (Yildirim and Hettiarachchy, 1998; Mahmoud and Savello, 1993). MTGase cross-linked films were stabilized by covalent bond (Yildirim and Hettiarachchy, 1998; Mahmoud and Savello, 1993). The cross-linked proteins in the surimi film generally tended to loss their solubility, especially with increasing MTGase. The losses in both film and protein solubility were in accordance the increase in EAB of the film added with MTGase (Table 15).

Table 17 Film solubility and protein solubility of bigeye snapper surimi film incorporated with palm oil as affected by MTGase levels.

MTGase (units/g protein)	Film solubility[#] (%)	Protein solubility[#] (%)
0 (control)	25.34±0.56[a]*	13.16±0.06[a]
5	23.65±0.06[b]	12.21±0.17[b]
10	22.55±0.25[c]	10.87±0.57[c]
20	22.11±0.36[c]	10.70±0.40[c]
40	21.47±0.40[d]	10.12±0.09[d]
80	19.54±0.17[e]	7.08±1.57[e]

[#]Mean±SD from three determinations.

*The different superscripts in the same column indicate the significant differences ($P < 0.05$).

5.2.5 Protein patterns of bigeye snapper surimi film

SDS-PAGE protein patterns of surimi films containing palm oil and added with different levels of MTGase (5, 10, 20, 40 and 80 units/ g protein) are depicted in Figure 13. SDS-PAGE study revealed that surimi films added with

MTGase consisted of the polymerized proteins as evidenced by the lowered protein band intensity, particularly MHC and actin. This aggregate might play a role in the increased EAB and decreased solubility of resulting film. From the study, protein aggregation induced by MTGase was conducted at pH 5.0, not pH 3.0 to avoid the inactivation of MTGase.

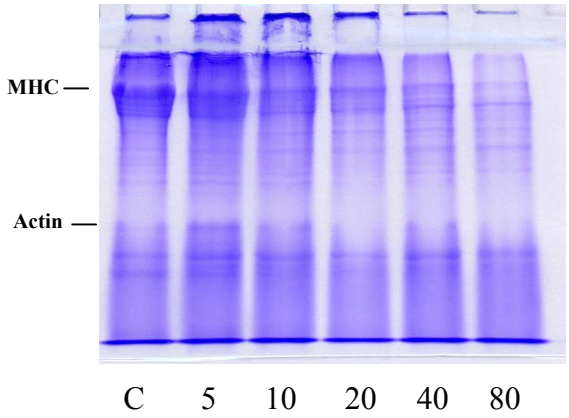


Figure 13 Protein patterns of bigeye anapper surimi films containing palm oil added with MTGase at different levels, C: control film (without MTGase). Numbers denote MTGase levels (units/ g protein).

From the result, bigeye snapper surimi films added with 5 mM formaldehyde had the highest mechanical property and lowest WVP, compared with other samples added with other aldehydes or MTGase. Therefore, bigeye snapper surimi films incorporated with 5 mM formaldehyde was chosen and used for further study.

5.2.6 Moisture sorption isotherms

Moisture sorption isotherms of the film samples were determined at 4ºC and room temperature (Figure 14). Films added with 5 mM formaldehyde had the moisture sorption isotherms as a sigmoid shape, which is in common with those of most foods and hydrophilic polymers. At low water activities (0.15-0.45), moisture content of the films kept at both 4ºC and room temperature increased slowly. Thereafter, moisture content of the films increased rapidly between Aw of 0.45-0.90 ($P>0.05$).

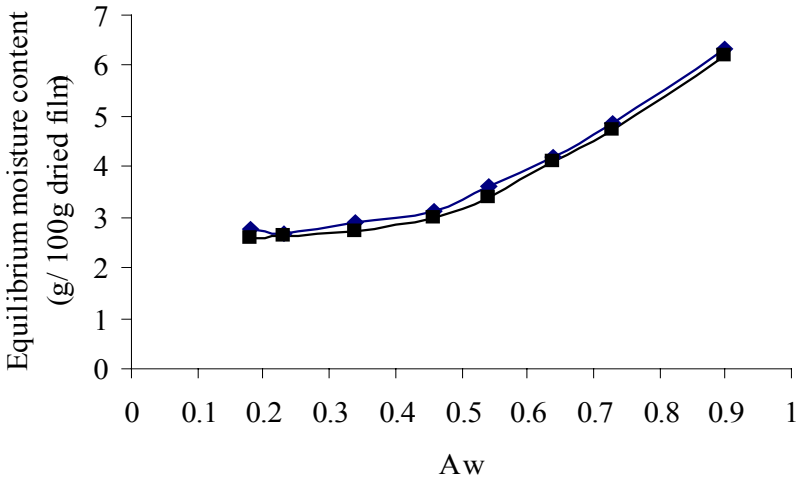


Figure 14 Moisture sorption isotherms of the film incorporated with palm oil and added with 5 mM formaldehyde at 4ºC (■) and room temperature (▲).

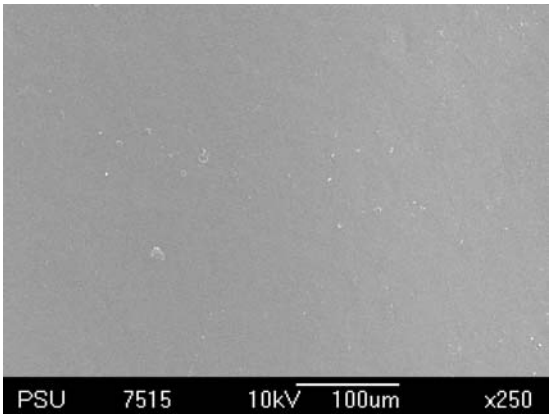
6. Characterization of bigeye snapper surimi film

6.1 Microstructure

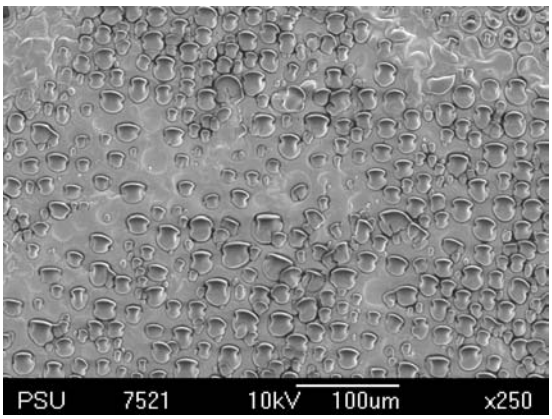
The SEM micrographs of surimi films without and with oil, either in the presence or absence of cross-linker are shown in Figure 15. The control film (without lipids and formaldehyde) had the smooth and continuous surface without grainy and porous structure. However, surface of surimi films added with palm oil at 75% glycerol substitution as well as films incorporated with palm oil at 75% glycerol substitution and 5 mM formaldehyde exhibited more protruding structure with oil droplet dispersion throughout the film (Figure 15). With addition of palm oil at 75% substitute of glycerol, the surface of surimi film was irregular with the distribution of oil droplets. The continuously spread oil droplets on the film surface might be associated with the reduced WVP of the film (Table 9).

The surface of films incorporated with oil and formaldehyde was not smooth and the grainy surface was obtained. Slightly larger oil droplet was found in the presence of formaldehyde. When formaldehyde fixed protein (Thies, 1995), larger protein aggregates could not function well as the emulsifier and could not imbed the oil droplet effectively. As a result, some coalescence might occurred as shown by the larger oil droplet. The greater cohesion of the polymeric matrix on the film surface together with the dispersed oil droplet might be associated with the reduced WVP and improved mechanical properties of film (Table 12). From the result, surface structural differences possibly illustrated the different properties of surimi films.

(a)



(b)



(c)

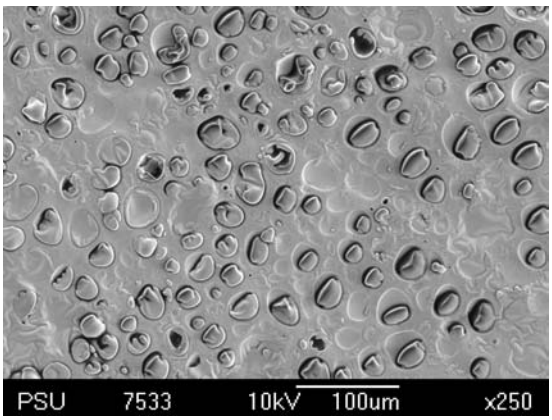


Figure 15 SEM micrographs of bigeye snapper surimi films. Control film (without lipid and formaldehyde) (a), film incorporated with palm oil at 75% glycerol substitution (b) and film incorporated with palm oil at 75% glycerol substitution and 5 mM formaldehyde (c).

6.2 Hydrolysis by protease

The protein digestibility of bigeye snapper surimi films are presented as the degree of hydrolysis (Table 18). The degree of hydrolysis of all samples was between 59.70 to 81.92%. The degree of hydrolysis of the control film (without palm oil and formaldehyde) was highest, compared with other samples. Hydrolysis of films by α-chymotrypsin markedly decreased when the palm oil and formaldehyde were incorporated ($P<0.05$). The films incorporated with palm oil at 75% glycerol substitution were hydrolyzed to a lower extent by α-chymotrypsin, compared with the control film. Distribution of oil droplets with high hydrophobicity could impede the interaction between substrate and enzyme. The lowest degree of hydrolysis of films incorporated with palm oil at 75% glycerol substitution and 5 mM formaldehyde suggested the resistance of films to enzymatic hydrolysis. The cross-links between proteins in surimi via chemical reaction through formaldehyde and amino acid side chain reactive groups (Ghorpade et al., 1995; Rhim et al., 2000; Parris and Coffin, 1997) could strengthen the material through formation of new covalent bonds (Gennadios and Weller, 1992), as indicated by the larger TS (Table 12). The larger aggregate could also be more resistant to hydrolysis by proteinases. However, surimi films incorporated with formaldehyde exhibited possible toxicity (Galietta et al., 1998). Therefore, these surimi films can be used as biodegradable films instead of edible film.

Table 18 Degree of hydrolysis (%) of bigeye snapper surimi films incorperated with oil and/or formaldehyde.

Film samples	degree of hydrolysis[#] (%)
Control**	81.92±0.50[a]*
Palm oil at 75 % glycerol substitution	71.46±0.44[b]
Palm oil at 75 % glycerol substitution + 5 mM formaldehyde	59.70±0.46[c]

** Films were prepared using 2% protein with 50% glycerol (based on protein content) at pH 3.0.
[#]Mean±SD from three determinations.
*The different superscripts in the same column indicate the significant differences ($P < 0.05$).

6.3 Protein solubility

Protein solubility of bigeye snapper surimi films in various solvents is shown in Table 19. The distribution and extents of inter- and intra-molecular interactions between proteins, which give rise to a three-dimentional network structure of the films, could affect their mechanical properties. The main associative forces involved in surimi films was reported to be hydrogen bonds, hydrophobic and ionic interactions (Shiku et al., 2004). Hydrogen bonds are considered important in contributing to the tensile strength of protein films (Meier, 1990). As shown in Table 19, proteins in the control film (without lipid and formaldehyde), films incorporated with palm oil at 75% glycerol substitution and films incorporated with palm oil at 75% glycerol substitution and 5 mM formaldehyde were soluble in S1 (20 mM Tris-HCl (pH 8.0)+1% (w/v) SDS), approximately 31.17, 31.38 and 22.93%, respectively. S1 has been reported to disrupt hydrogen bonds stabilizing the film matrix. The solubility of all samples markedly increased to more than 50% for the control film and films incorporated with palm oil at 75% glycerol substitution. When dissolved with

S2, 71.03% of protein in films incorporated with palm oil at 75% glycerol substitution and 5 mM formaldehyde were obtained. With the addition of 8.0 M Urea (S2), hydrophobic interactions can be destroyed. The addition of 2% βME to S2 (S3) did not increase the protein solubility of the control film and films incorporated with palm oil at 75% glycerol substitution ($P>0.05$). The increase in solubility of films incorporated with palm oil at 75% glycerol substitution and 5 mM formaldehyde, approximately 23.76%, was noticeable. The result indicated that disulfide bonds were not formed in the control film and films incorporated with palm oil at 75% glycerol substitution, while disulfide bonds occurred in films incorporated with palm oil at 75% glycerol substitution and 5 mM formaldehyde. Increase in protein solubility in the presence of 2% βME suggested that the formation of disulfide bonds took place as the new covalent bonds between proteins in bigeye snapper surimi film. The chemical reaction through formaldehyde and amino acid side chain reactive groups might induce conformational changes of protein molecules, in the way which favored the oxidation of sulfhydryl groups of proteins. Surimi film was formed by the cross-links stabilized by various bonding including hydrogen bond, hydrophobic interaction as well as disulfide bond (Shiku et al., 2003). From the results, it was elucidated that hydrogen bonds and hydrophobic interactions played an important role in the formation of bigeye snapper surimi films and disulfide bond could be formed in the presence of formaldehyde.

Table 19 Protein solubility (%) of bigeye snapper surimi films in various solvents.

Film samples	Solutions		
	S1*	S2	S3
Control***	31.17±1.45[a]**	55.83±0.39[c#]	55.84±0.48[c]
Palm oil at 75 % glycerol substitution	31.38±1.37[a]	57.88±0.28[b]	57.94±0.35[b]
Palm oil at 75 % glycerol substitution + 5 mM formaldehyde	22.93±1.61[b]	71.03±0.60[a]	94.79±0.64[a]

*** Films were prepared using 2% protein with 50% glycerol (based on protein content) at pH 3.0
*S1: 20 mM Tris-HCl (pH 8.0)+1% (w/v) SDS; S2: 20 mM Tris-HCl (pH 8.0) +1% (w/v) SDS+8.0 M Urea; S3: 20 mM Tris-HCl (pH 8.0)+1% (w/v) SDS+8.0 M Urea +2% βME.
#Mean±SD from three determinations.

**The different superscripts in the same column indicate the significant differences ($P < 0.05$).

6.4 Thermal properties

Typical DSC thermograms of the control film, films incorporated with palm oil at 75% glycerol substitution as well as films incorporated with palm oil at 75% glycerol and 5 mM formaldehyde are depicted in Figure 16 and 17. The abrupt change in the slope of heat flow curves of films reveals their glass transition temperature, T_g. From the result, T_g of films shifted to the higher temperature when oil was incorporated in the absence and presence of formaldehyde, respectively (Table 20). This indicated that oil and formaldehyde incorporation decreased flexibility of the protein molecules in the film matrix of bigeye snapper surimi film. An increase in the T_g associated with the cross-linking and palm oil addition was observed.

The highest T_g value observed in the films incorporated with palm oil at 75% glycerol and 5 mM formaldehyde was associated with the greatest resistance to break and the lowest elongation of the resulting film. This might be due to a lower molecular mobility or plastic character of formaldehyde cross-linking (Hermandez-Munoz et al., 2004). T_g of proteins increases with the chain rigidity and the intensity of both inter- and intramolecular interactions, including hindrance to internal rotation along the macromolecular chain (Barreto et al., 2003). T_g of films can vary depending on the composition in films, plasticizer, lipid and the cross-linker (Audic and Chaufer, 2005). From the first DSC scan, the lower T_g observed in the control film compared to the films modified with oil and/or formaldehyde might also contribute from the presence of higher residual water absorbed in the film sample (Table 20). Water (T_g of -135 °C (Johari et al., 1987)) can exhibit plasticizing effect, T_g depression, in polymeric film especially hydrophilic film (Roos, 1995; Arvanitoyannis and Biliaderis, 1998; Talja and Roos, 2001; Sorbal et al., 2002). The water plasticization resulted from an increase in the free volume of the material allowing a higher molecular mobility. On the respective DSC thermograms of the second scan, the glass transitions of film samples were more pronounced and shifted to lower temperatures compared to those of the first scan. The higher T_g's of films observed in the first scan might result from the presence of water in the samples. At below 0 °C, ice crystals might impede the mobility of the protein molecules especially the region closed proximity to the crystal boundaries or constrained between the neighboring crystals. In addition, there was no melting transition observed in surimi protein films, suggesting highly aggregated or cross-linked structure.

Thermal degradation behavior of the polymeric film can be studied by using thermogravimetric analyzer (TGA). Degradation behavior of the control films, films incorporated with palm oil at 75% glycerol substitution as well as films incorporated with palm oil at 75% glycerol and 5 mM formaldehyde are shown in Figure 18. The film samples plasticized with glycerol showed two-stage degradation (Figure 18). Corresponding degradation temperatures (T_d) are shown in Table 21. The control films had lowest 1st-stage T_d, compared with other film samples. The 2nd-stage T_d's of the films were not significant different, even though the highest one was observed for the formaldehyde cross-linked film. From the result, it was presumed that first degradation temperature of plasticized film samples possibly reflected the degradation of small molecules such as plasticizer and cryoprotectants or side chain of protein. Second degradation was mainly associated with the degradation of the protein component. Noted that the surimi film without plasticizer and cryoprotactant showed single degradation at about 311 °C (Table 21). From the result, the decomposition of films was not completed when heated up to 500 °C as evidenced by the remaining weight (approximately 20%). This suggested the presence of highly cross-linked network which stabilized the structure. From these data, it was suggested the increase in heat stability of bigeye snapper surimi films when oil was incorporated with or without formaldehyde as indicated by the higher T_g and T_d. Therefore, the differences in film compositions most likely affected thermal properties of resulting film differently.

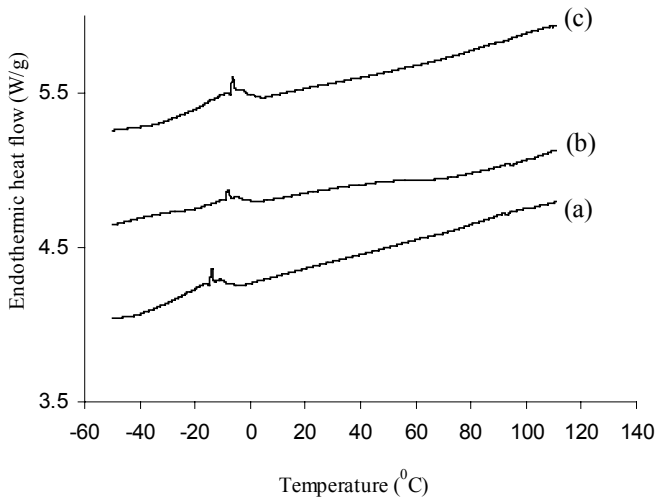


Figure 16 First scan of DSC thermogram of (a) the control film, (b) film incorporated with palm oil at 75 % glycerol substitution and (c) film incorporated with palm oil at 75 % glycerol and 5 mM formaldehyde. Film were prepared using 2% protein and 50% glycerol (based on protein content) at pH 3.0.

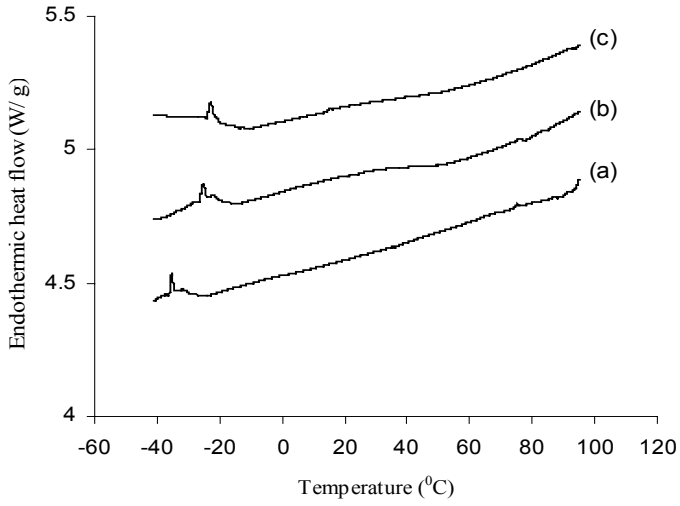


Figure 17 Second scan of DSC thermogram of (a) the control film, (b) film incorporated with palm oil at 75 % glycerol substitution and (c) film incorporated with palm oil at 75 % glycerol and 5 mM formaldehyde. Film were prepared using 2% protein and 50% glycerol (based on protein content) at pH 3.0.

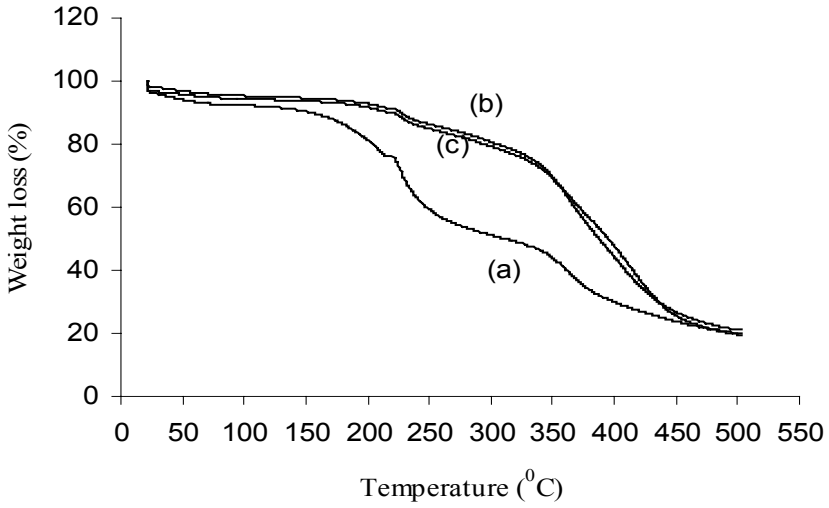


Figure 18 TGA thermogram of (a) the control film, (b) film incorporated with palm oil at 75 % glycerol substitution and (c) film incorporated with palm oil at 75 % glycerol and 5 mM formaldehyde. Film were prepared using 2% protein and 50% glycerol (based on protein content) at pH 3.0.

Table 20 Glass transition temperature (T_g) of the control films, films incorporated with palm oil at 75 % glycerol substitution, films incorporated with palm oil at 75 % glycerol and 5 mM formaldehyde.

Film samples	Moisture content (%)	T_g (°C)	
		1st scan	2nd scan
Control (plasticized)	9.20±0.02[a]	-17.2±0.25[c]	-32.60±0.02[c]
Palm oil 75%glycerol substitution	6.01±0.01[b]	-10.60±0.02[b]	-27.10±0.02[b]
Palm oil 75%glycerol substitution + 5 mM formaldehyde	4.21±0.02[c]	-8.00±0.06[a]	-24.50±0.02[a]

*** Films were prepared using 2% protein with 50% glycerol (based on protein content) at pH 3.0.
[#]Mean±SD from three determinations.

**The different superscripts in the same column indicate the significant differences ($P < 0.05$)

Table 21 Degradation temperature (T_d) of the control films, films incorporated with palm oil at 75 % glycerol substitution, films incorporated with palm oil at 75 % glycerol and 5 mM formaldehyde and surimi (unplasticized, without cryoprotectant).

Film samples	T_d (°C)	
	1st degradation	2nd degradation
Control (plasticized)	185.70±2.26[c]	334.80±4.50[b]
Palm oil 75%glycerol substitution	209.70±5.09[b]	337.20±1.37[b]
Palm oil 75%glycerol substitution + 5 mM formaldehyde	215.50±1.28[b]	340.00±2.14[ab]
Surimi (unplasticized, without cryoprotectant)	311.10±3.56[a]	-

*** Films were prepared using 2% protein with 50% glycerol (based on protein content) at pH 3.0.
[#]Mean±SD from three determinations.
*The different superscripts in the same column indicate the significant differences ($P < 0.05$).

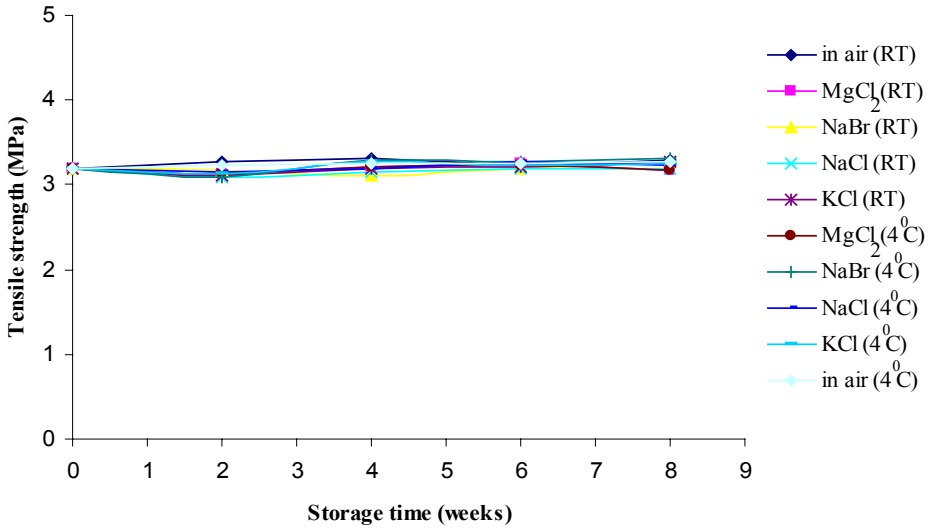
7. Changes of films during storage

7.1 Changes in mechanical and water vapor barrier properties

Bigeye snapper surimi films containing palm oil at 75% glycerol substitution and 5 mM formaldehyde were stored under varying RHs using different saturated salt solutions including $MgCl_2$, NaBr, NaCl or KCl at room temperature and 4ºC. Film samples were also stored in air at both temperatures. No changes in mechanical properties and water vapor barrier properties were found throughout the storage at both storage temperatures (Figure 19, 20). Therefore, bigeye snapper surimi

films displayed high stability regardless of storage time, temperature and RH studied. The result was in agreement with Cuq et al. (1996) who reported that solubility in water, WVP and mechanical properties of myofibrillar protein based-films remained constant for 8 weeks at 20ºC and 58%RH. In contrast, Park et al. (1994) observed a lowering of elongation values for a wheat gluten film monitored during 20 days of storage at 25ºC and 50% RH, which could be due to migration of glycerol (the plasticizing agent) from the bulk of the film to the surface. Somanathan et al. (1992) reported that triethanolamine-treated casein films became considerably less resistant after 1 year of storage at 25ºC and 65% RH. Soy protein isolate film underwent different changes during storage at 25ºC depending on the water content in the container (Park and Hettiarachchy, 2000). The degree of degradation of soy protein isolate films was greater at 25ºC than at 15ºC. The degradability of the soy protein isolate films during storage appeared to be more sensitive to moisture than to temperature. From the result, the formaldehyde added in conjunction with palm oil might be of assistance in stabilizing the film network in which the oil droplets were dispersed. Oil dispersed could prevent the water migration into the film, while formaldehyde cross-linked proteins might be resistant to mechanical forces. As a consequence, films were stable throughout the storage of 8 weeks.

(A)



(B)

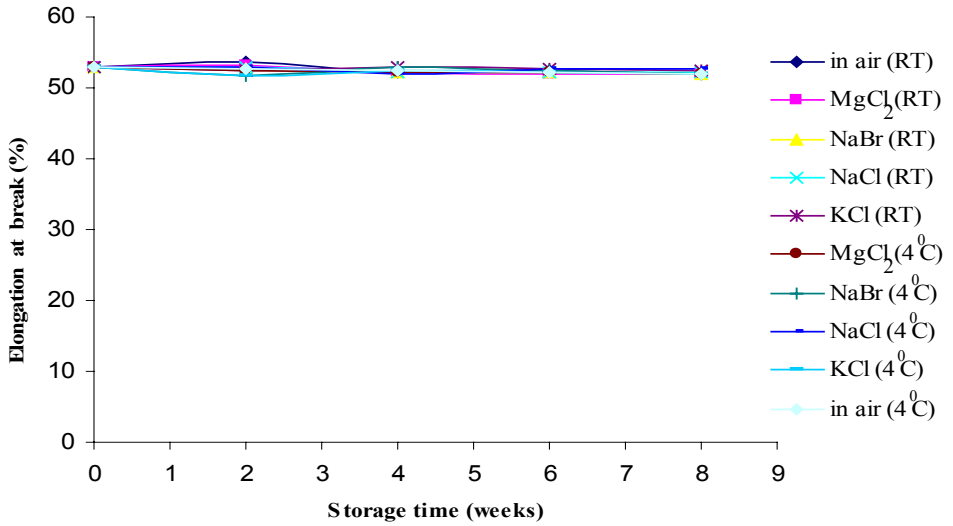


Figure 19 Changes in tensile strength (A) and elongation at break (B) of bigeye snapper surimi films containing palm oil at 75% glycerol substitution and 5 mM formaldehyde during storage at different temperatures and RHs. RT: room temperature.

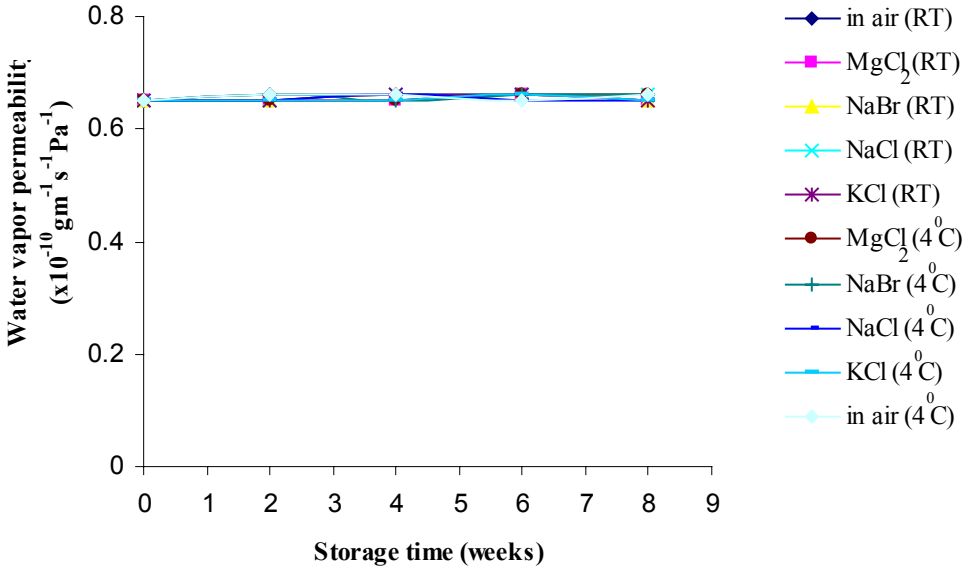


Figure 20 Changes in water vapor permeability of bigeye snapper surimi films containing palm oil at 75% glycerol substitution and 5 mM formaldehyde during storage at different temperatures and RHs. RT: room temperature.

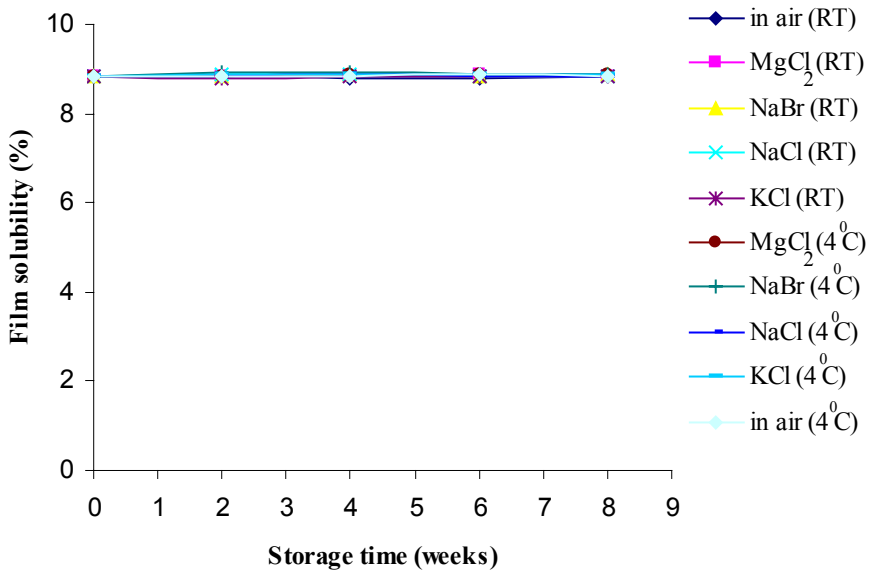
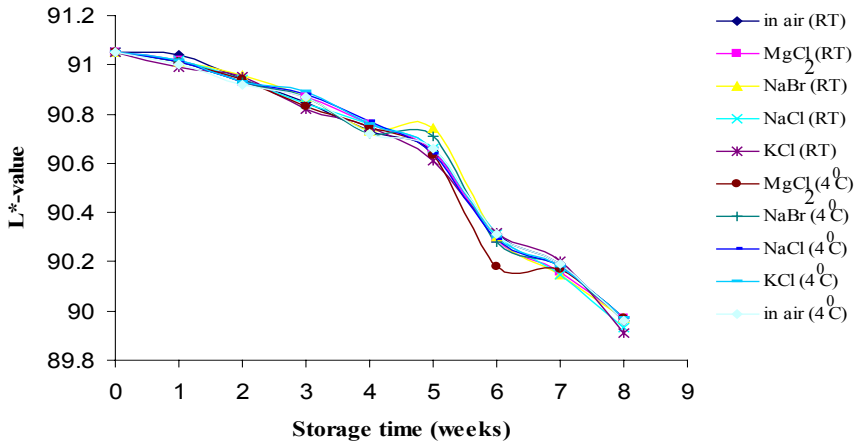


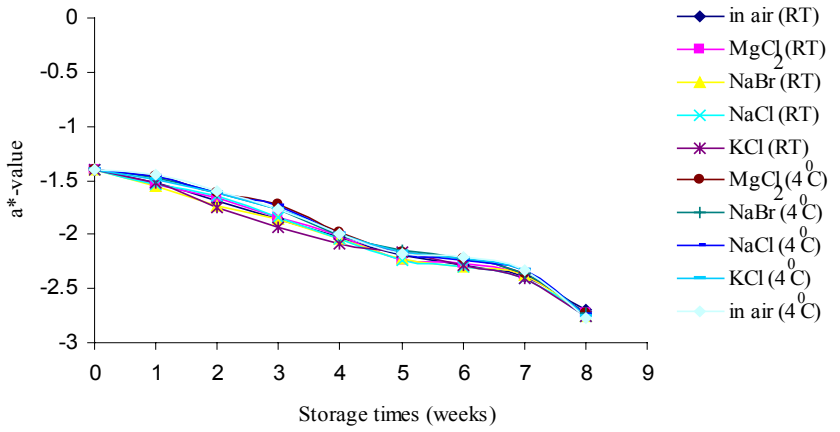
Figure 21 Changes in film solubility of bigeye snapper surimi films containing palm oil at 75% glycerol substitution and 5 mM formaldehyde during storage at different temperatures and RHs. RT: room temperature.

7.2 changes in color and transparency

The color and transparency of bigeye snapper surimi film containing palm oil and formaldehyde during the storage are shown in Figure 22, 23. All film samples became more darker as evidenced by the decrease in L*- and a*-values when the storage time increased ($P<0.05$) (Figure 22). The increases in both b*-value and transparency value were observed in films stored for a longer time ($P<0.05$). The results suggested that the films were more yellowish with increasing storage time. The increase in transparency value indicated the increase in opacity, possibly caused by the greater orientation of the polymeric matrix in the protein network. The increase in b*- value, associated with the formation of yellow hue, could be the result of nonenzymatic browning reactions. Acid process used for film-forming solution preparation might induce the hydrolysis of sucrose added in surimi as cryoprotectant. As a result, the free reducing sugars were formed and underwent the browning reaction with amino groups in surimi, especially with increasing storage time. Carbonyl groups of formaldehyde added might serve as the reaction group for Maillard reaction. In general, the yellow/brown coloration associated with protein-aldehyde interactions is due to the various intermediate or final products of the Maillard reaction (Cheftel et al., 1985; Damodaran, 1996). The result was in agreement with Somanathan et al. (1992) who reported that triethanolamine-treated casein films became brown in color after 1 year of storage at 25ºC and 65% RH. Cuq et al. (1996) also found the increase in b*-value of fish myofibrillar protein-based film formulated with saccharose as a plasticizer. From the result, storage temperature and

RH did not show the effect on the color and transparency of films during extended storage.





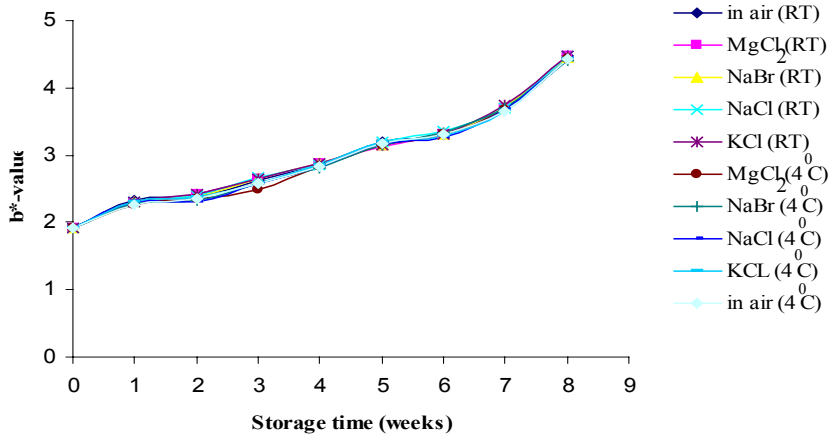


Figure 22 Changes in color of bigeye snapper surimi films containing palm oil at 75% glycerol substitution and 5 mM formaldehyde during storage at different temperatures and RHs. RT: room temperature.

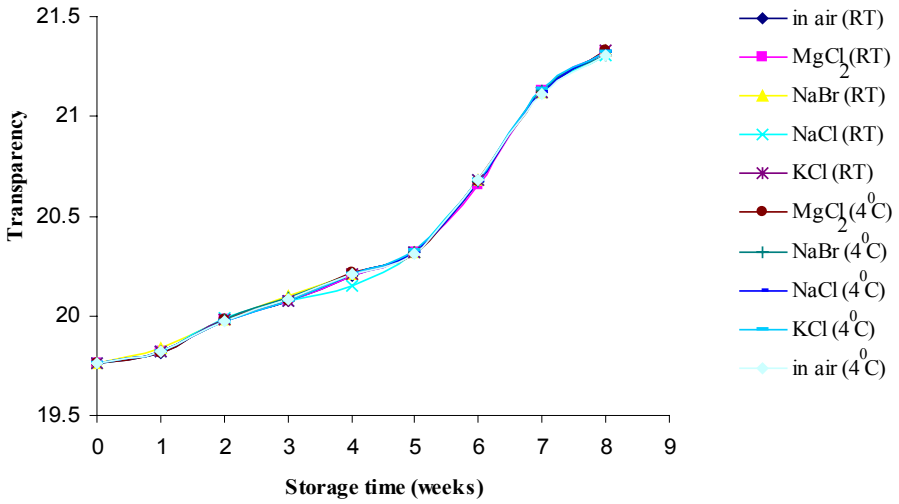


Figure 23 Changes in transparency of bigeye snapper surimi films containing palm oil at 75% glycerol substitution and 5 mM formaldehyde during storage at different temperatures and RHs. RT: room temperature.

8. Uses of bigeye snapper surimi film to extend the shelf-life of dried fish powder

Dried fish powder was placed in the bottles and bigeye snapper surimi film was placed on the bottle and sealed with grease before tightening with the silicone bands. The fish powder was sampled and analyzed during storage.

8.1 Change in moisture content

Moisture content of dried fish powder of all samples increased as the storage time increased ($P<0.05$) (Figure 24). After 15 days of storage, samples packaged with bigeye snapper surimi films incorporated with palm oil at 75% glycerol substitution (section 7) and films incorporated with palm oil at 75% glycerol substitution and 5 mM formaldehyde (section 8) and polyethylene plastic films

(LDPE) had the increase in moisture content by 3.08, 2.59 and 3.60-fold, respectively. This result suggested that films incorporated with palm oil and formaldehyde showed the highest water vapor barrier property, compared with other films. The stability of food and their resistance to oxidation is a function of moisture content (Labuza, 1971). Most biochemical reactions are accelerated by increasing moisture content, but there are others for which the speed of reaction is greatest at low levels of relative humidity. For instance, auto-oxidative processes are particularly favored by very low moisture content (about 20%). In addition to its activity as a solvent and reactant, water can participate directly in hydrolytic cleavage, the products of which can participate in nonenzymatic browning (Pomeranz, 1991). From the result, bigeye snapper surimi film incorporated with palm with or without 5 mM formaldehyde exhibited the superior water vapor barrier property to PE film.

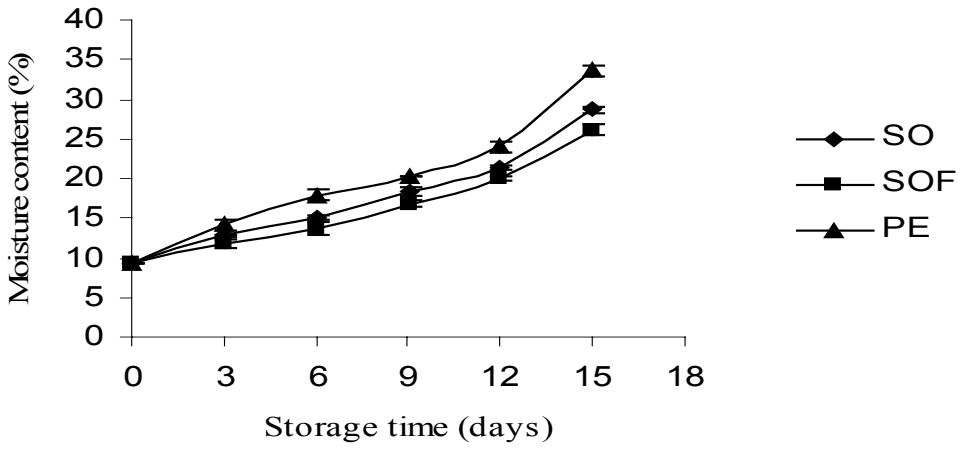


Figure 24 Changes in moisture content of dried fish powder packaged using bigeye snapper surimi films incorporated with palm oil at 75% glycerol substitution (SO), bigeye snapper surimi films incorporated with palm oil at 75% glycerol substitution and 5mM formaldehyde (SOF) or

polyethylene film (PE) during storage at 30-32ºC. Bar indicates standard deviation from triplicate determinations.

8.2 Changes in color

L*, a* and b*-values of dried fish powder packaged with three different films are shown in Figure 25. In general, the decrease in L*-value and increase in a*- and b*-values were observed in fish powder throughout the storage of 15 days. Samples packaged in surimi film incorporated with palm oil at 75% glycerol substitution and 5 mM formaldehyde had the higher lightness and lower redness and yellowness as indicated by higher L*-value and lower a*- and b*-values, respectively ($P<0.05$). From the result, the increased b*-values indicated the formation of yellowish pigment, especially via Maillard reaction, which might be associated with increasing moisture content in dried fish powder. The increase availability of water accelerates nonenzymatic browning reactions (Pomeranz, 1991). Therefore, the retardation of moisture migration through the bigeye snapper surimi film containing oil and formaldehyde could impede the browning reaction, which might be associated with lipid oxidation.

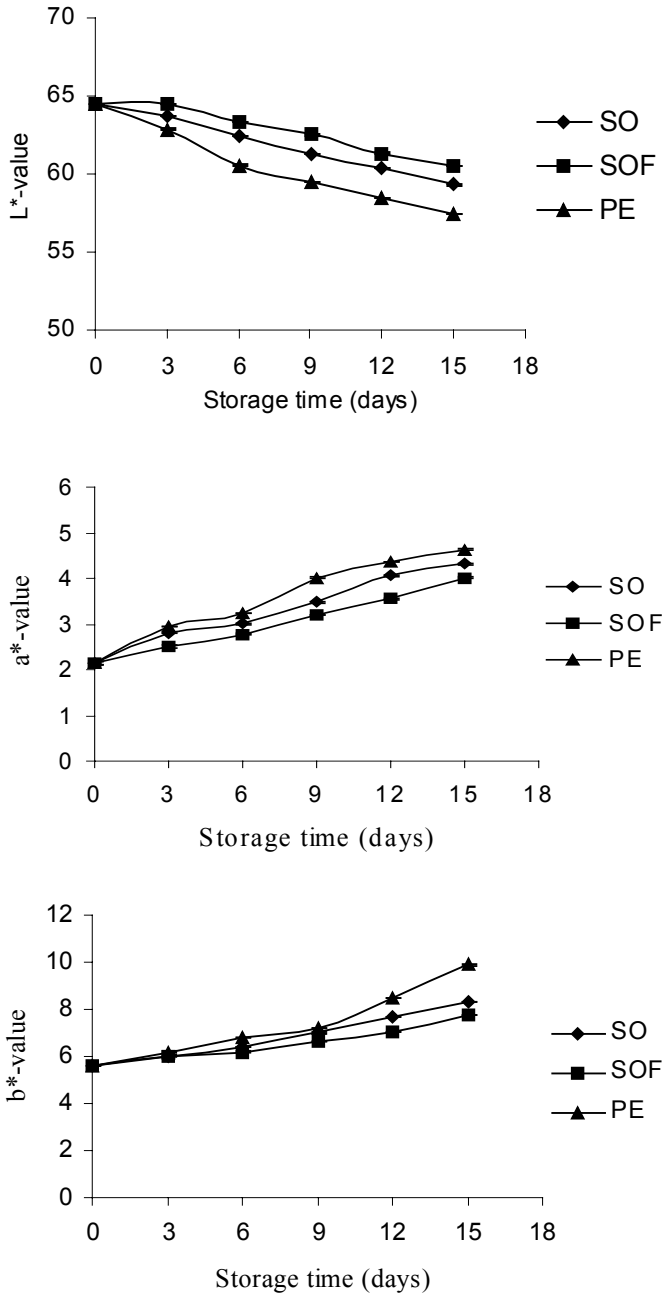


Figure 25 Changes in color of dried fish powder packaged using bigeye snapper surimi films incorporated with palm oil at 75% glycerol substitution (SO), bigeye snapper surimi films incorporated with palm oil at 75% glycerol substitution and 5mM formaldehyde (SOF) or polyethylene film (PE) during storage at 30-32ºC. Bar indicates standard deviation from triplicate determinations.

8.3 Change in lipid oxidation

TBARS values of dried fish powder packaged using different films during the storage are shown in Figure 26. TBARS values of all samples increased continuously with increasing storage time ($P<0.05$). Generally, samples packaged using surimi film incorporated with oil and formaldehyde had the lower TBARS value, indicating the lower lipid oxidation. After 15 days of storage, TBARS value of samples packaged in surimi film incorporated with oil, surimi film incorporated with oil and formaldehyde and polyethylene plastic films increased by 3.17, 2.66 and 3.77-fold, respectively. Due to the high content of polyunsaturated fatty acid in the fish muscle, fish is much more susceptible to oxidation (Xiong, 1997; Saeed and Howell, 2002). The double bonds in an unsaturated fatty acid are locked into position when oxygen reacts with the methylene group adjacent to the double bonds (Frankel, 1985). However, oxidation was retarded when surimi film containing palm oil either with or without formaldehyde were used. This result implied that the oxygen permeability of surimi film might be lower than PE film. The result was in agreement with Cuq (2002) who reported that oxygen permeability of a dry fish myofibrillar protein-based films was lower by three orders of magnitude than that of low-density polyethylene. Thus myofibrillar protein-based films can be used for protecting fish or meat pieces from oxidation or dehydration during storage. Protein-based films were the excellent oxygen and carbondioxide barriers (Kester and Fennama, 1984; Kester and Fennama, 1989; Gennadios et al., 1993). The increase in TBARS might be associated with the increase in the carbonyl groups of aldehydes. Those carbonyl groups might react with amino groups of proteins in dried fish powder via Maillard reaction. Thus, the

browning was enhanced via Maillard reaction as shown by the increased yellowness (b*-value) (Figure 25).

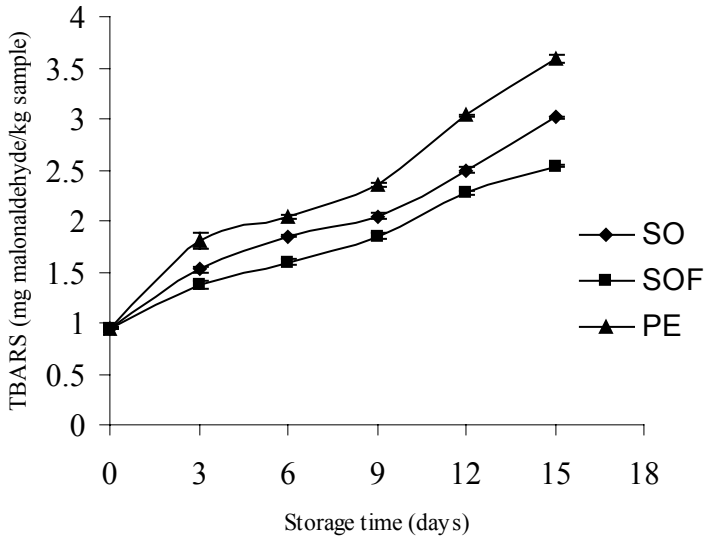


Figure 26 Changes in TBARS of dried fish powder packaged using bigeye snapper surimi films incorporated with palm oil at 75% glycerol substitution (SO), bigeye snapper surimi films incorporated with palm oil at 75% glycerol substitution and 5mM formaldehyde (SOF) or polyethylene film (PE) during storage at 30-32ºC. Bar indicates standard deviation from triplicate determinations.