Comparative study on chemical and gel-forming properties of surimi from freshwater and marine fish during frozen storage

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Abstract

Consumption of surimi based-products has been increasing, while marine raw materials, particularly tropical fish, continuously decrease due to overexploitation of fishing resources. Freshwater fish have been shown to provide good surimi gel. However, intrinsic properties of freshwater and marine surimi have not been fully realized. This study aimed at comparing the frozen stability of surimi produced from freshwater and marine fish. Surimi from three freshwater fish species including silver carp (*Hypophthalmichthys molitrix*), rohu (*Labeo rohita*), tilapia (*Oreochromis niloticus*) and marine fish, threadfin bream (*Nemipterus* spp.), were produced at a commercial surimi plant. Samples were kept at -20 °C and analyzed at various time intervals of 0, 3, 6 and 9 months. All surimi samples were prepared without addition of egg white powder. The results showed that protein solubility of all samples decreased continuously throughout the frozen storage. After 9 months of frozen storage, solubility of surimi made from freshwater fish decreased by 52-57%, while only 30% reduction was observed in threadfin bream. Silver carp surimi showed the highest gel-forming ability as compared to other freshwater species studied. Gel quality from all freshwater fish surimi decreased throughout 9-month storage with a 30 and 20% decrease in breaking force and deformation, respectively. Breaking force of threadfin bream surimi only decreased by 9% after 9 months without a significant change of deformation. No marked change in whiteness of all species during frozen storage. In addition, actomyosin conformation of all fish species slightly changed during frozen storage. Changes of reactive sulfhydryl group contents and surface hydrophobicity indicated that muscle proteins of freshwater fish were sensitive to frozen denaturation to a greater extent than that of threadfin bream surimi.

Keywords: surimi, frozen storage, freshwater fish, denaturation, gel-forming ability

1. Introduction

Surimi is the wet concentrate of the myofibrillar proteins of fish muscle that is mechanically deboned, water washed and frozen. Surimi is typically produced from various species of marine fish

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However, increased consumption of surimi-based products and continous overexploitation of marine tropical fishing resources have led tropical surimi manufacturers to search for alternative resources. Tropical fish used in surimi production in Southeast Asia are mainly threadfin bream, bigeye snapper, croakers, and lizardfish. Among aquacultured freshwater fish, silver carp (*Hypophthalmichthys molitrix*) and tilapia (*Oreochromis niloticus*), have gained great attention because of their large production in countries like China, Thailand, Bangladesh, India, Iran, and Russian Federation (Asgharzadeh et al., 2010).

Surimi is typically kept frozen, resulting in frozen denaturation of myofibrillar protein and loss in protein functionality, especially gel-forming ability. Gel-forming ability of muscle proteins from threadfin bream, bigeye snapper, lizardfish, croaker and tilapia decreased during frozen storage (Benjakul et al., 2005; Zhou et al., 2006). Extended frozen storage caused protein denaturation, but the degree of changes would likely be species dependent. The information about the tropical freshwater fish as surimi raw material is very scanty. Changes in chemical and gel-forming ability during frozen storage of surimi produced from freshwater fish have not been clearly reported. Therefore, the purpose of the study was to investigate the chemical changes and gel-forming ability of surimi from four fish species, namely silver carp (*Hypophthalmichthys molitrix*), rohu (*Labeo rohita*), tilapia (*Oreochromis niloticus*) and threadfin bream (*Nemipterus* spp.) during frozen storage.

2. Materials and Methods

2.1 Chemicals

Bovine serum albumin (BSA) and ethylenediaminetetraacetic acid (EDTA) were purchased from Fluka (Buch, Swizerland). Trichloroacetic acid was purchased from BDH Chemicals Ltd (Poole, UK). Adenosine 5'-triphosphate (ATP), 1 anilino-8-napthalenesulfonate (ANS) and 5, 5'-dinitrobis (2-nitrobenzoic acid) were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other reagents and chemicals were of analytical grade.

2.2 Materials

Surimi from four fish species including silver carp (*Hypophthalmichthys molitrix*), rohu (*Labeo rohita*), tilapia (*Oreochromis niloticus*) and threadfin bream (*Nemipterus* spp.) were obtained from a surimi plant in Samutsakorn, Thailand. Samples were packed in polystyrene foam box and

transported to Suranaree University of Technology laboratory and kept at -20°C. Samples were taken for analysis at various time intervals of (0, 3, 6 and 9 months).

2.3 Salt soluble protein

Surimi was homogenized with 0.6 M NaCl, 20 mM Tris-maleate (pH 7.0) at a ratio of 1:9 (w/v). The homogenates were centrifuged at 8,000 x g (Sorvall Legend MACH 1.6/R, Thermo Electron LED Gmbh, Lengensellbold, Germany) at 4°C for 20 min. The supernatant was collected and protein extractability was determined by Bradford method using bovine serum albumin (BSA) as a standard (Bradford, 1976). This sample was referred to as protein solution.

2.4 Ca^{2+}-ATPase activity

ATPase activity was determined using the modified method described by Chomnawang et al. (2007). Protein solution (500 µl) was added to 250 µl of 0.6 M Tris-maleate (pH7.0) and 250 µl of 0.1 M $CaCl_2$. To the mixture, and deionized water was then added to a total volume of 3.75 ml. The reaction was incubated for 5 min at 25°C and 250 µl of 20 mM ATP (adenosine 5'-triphosphate) was added and incubated for exactly 8 min at 25°C. The reaction was stopped by adding 2.5 ml of chilled 15% (w/v) trichloroacetic acid. The reaction mixture was then centrifuged at 3,000 x g for 10 min, and the inorganic phosphate liberated in the supernatant was measured using a spectrophotometer. The absorbance was measured at 640 nm. Specific activity was expressed as moles inorganic phosphate (Pi) released/mg protein/min.

2.5 Reactive sulfhydryl (SH) groups

Reactive SH groups were determined using the modified method described by Yongsawatdigul and Park (2003). To 1 ml of protein solution (4 mg/ml), 9 ml of buffer containing 50 mM sodium phosphate buffer, 10 mM ethylenediaminetetraacetic acid, 0.6 M NaCl and 8 M urea (pH 7.0) were added. To 4 ml of the resultant mixture, 0.4 ml of 0.1% 5, 5'-dinitrobis (2-nitrobenzoic acid) was added. The mixture was incubated at 4°C for 1 h. The absorbance of the mixture was measured at 412 nm and reactive SH groups were calculated using the extinction coefficient of 13,600 $M^{-1}cm^{-1}$.

2.6 Surface hydrophobicity

Surface hydrophobicity (So) of sample was determined using a hydrophobic fluorescence probe, 1 anilino-8-napthalenesulfonate (ANS), according to Hayakawa and Nakai (1985). Protein solution (1 mg/ml) was diluted with 0.6 M NaCl, 20 mM sodium phosphate buffer (pH 7.0) to obtain a series of protein concentrations, from 0-1 mg/ml. To 2 ml of each protein solution, 10 µl of 8 mM ANS in 0.1 M sodium phosphate buffer (pH 7.0) was added and mixed well. Samples were kept in the dark for 10 min. Fluorescence intensity of the mixture was measured using a spectrofluorometer at excitation and emission wavelengths of 374 and 485 nm, respectively. The initial slope of the plot of fluorescence intensity versus protein concentration (% w/v) was referred to as So-ANS.

2.7 Gel preparation

Frozen samples were tempered at room temperature for 1 h. Samples from all treatments were mixed with salt and ice to obtain 2% NaCl and 78% moisture content. Sample was chopped in a Stephan vacuum cutter for 6 min and final chopping temperature was $\leq 10^{\circ}C$. The paste was stuffed into 30 mm-diameter cellulose casing and pre-incubated at $25^{\circ}C$ for 1 h, $40^{\circ}C$ and $65^{\circ}C$ for 30 min and followed by $90^{\circ}C$ for 30 min. Samples without pre-incubation were also prepared by heating at $90^{\circ}C$ for 30 min. After heating the gels were immediately chilled in ice water for 20 min and kept at $4^{\circ}C$ overnight before texture and color measurement.

2.8 Textural and color measurement

The chilled gels were left at room temperature for 2 h before texture measurement. Textural properties of gels were measured using a Texture Analyzer (TA-XT2 Stable Micro System, Surrey, U.K.) equipped with a 5-mm diameter spherical plunger probe. Gel samples were cut into pieces of 30 mm length. Penetration test was performed at a probe speed of 60 mm/min. Breaking force (g) and deformation (mm) were recorded. Color values (L*, a*, b*) of all samples were measured using a Minolta colorimeter (Konica Minolta, Japan). Whiteness of gel was calculated using the equation L* - 3b* (Park et al., 1994).

2.9 Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of mean was carried out by Duncan's multiple range test (DMRT). Analysis was performed using a SPSS package (SPSS for Windows, version 17.0 SPSS Inc., Chicago, Ill., U.S.A.).

3. Results and Discussion

3.1 Salt soluble protein

Protein solubility of all samples gradually decreased throughout 9 months of frozen storage (Fig. 1). The lowest solubility was observed in rohu surimi, especially at 9 months of frozen storage. After 9 months of frozen storage, protein solubility of rohu was decreased by 57%, while protein solubility of surimi from nile tilapia, silver carp and threadfin bream were decreased by 54, 52 and 30%, respectively. A decrease in protein solubility indicated that aggregation as well as denaturation of proteins caused by frozen storage. Threadfin bream muscle showed the highest frozen stability among fish studied.

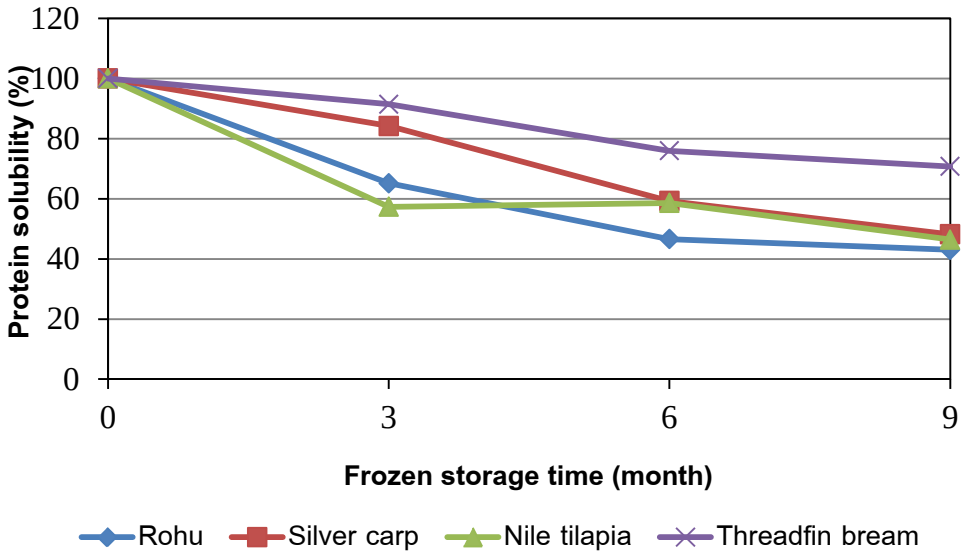

Figure 1. Changes in protein solubility of surimi prepared from four fish species during frozen storage at -20°C for 9 months.

3.2 Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity of surimi from all species increased continuously throughout 9 months of frozen storage (Fig. 2). Ca^{2+}-ATPase activity is a measure of muscle tissue ability to hydrolyze adenosine triphosphate (ATP) in the presence of Ca^{2+} ions. The myofibrillar adenosine

triphosphatase (ATPase) is located in the myosin head region (Chan et al., 1995). Thus, Ca^{2+}-ATPase activity is a good indicator of the integrity of myosin molecule (Roura and Crupkin, 1995). Yongsawatdigul and Park (2002) reported that a decrease of Ca^{2+}-ATPase activity implied conformational changes of myosin. In this study, extracted myofibrillar protein content and ATP hydrolysis decreased during frozen storage. But a decrease of protein content was greater than the ability to hydrolyze ATP, leading to an increase in Ca^{2+}-ATPase activity during frozen storage. This result indicated that integrity of extractable myosin head of these tropical fish was not greatly affected by frozen storage up to 9 months.

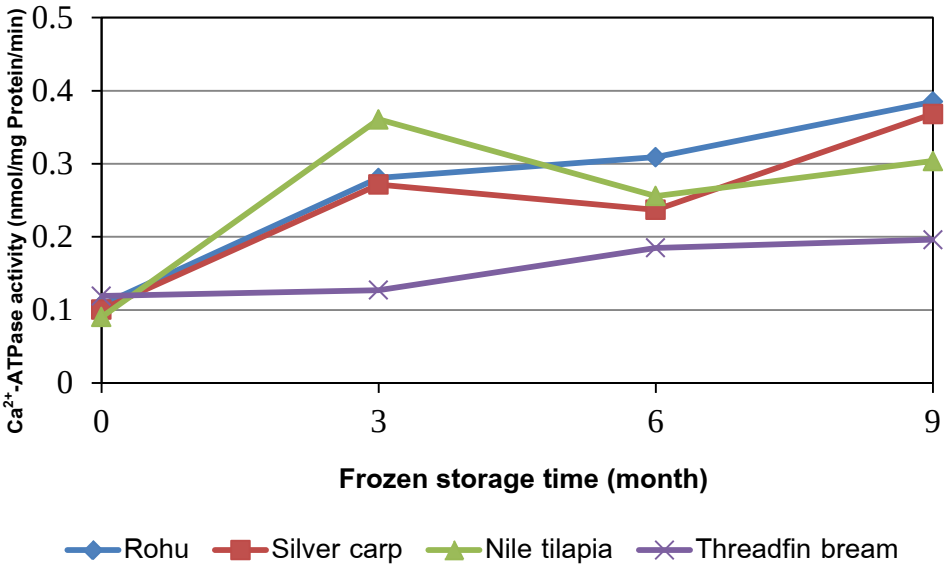

Figure 2. Changes in Ca^{2+} -ATPase activity of surimi from four fish species during frozen storage at -20°C for 9 months.

3.3 Reactive sulfhydryl (SH) groups

Reactive SH content of surimi from freshwater fish increased after 9 months of storage ($P<0.05$), while that of threadfin bream remained constant ($P<0.05$) (Fig. 3). An increase in the reactive SH content indicated unfolding of actomyosin. This result suggested that the stability of the freshwater fish muscle proteins seemed to be lower than that of threadfin bream.

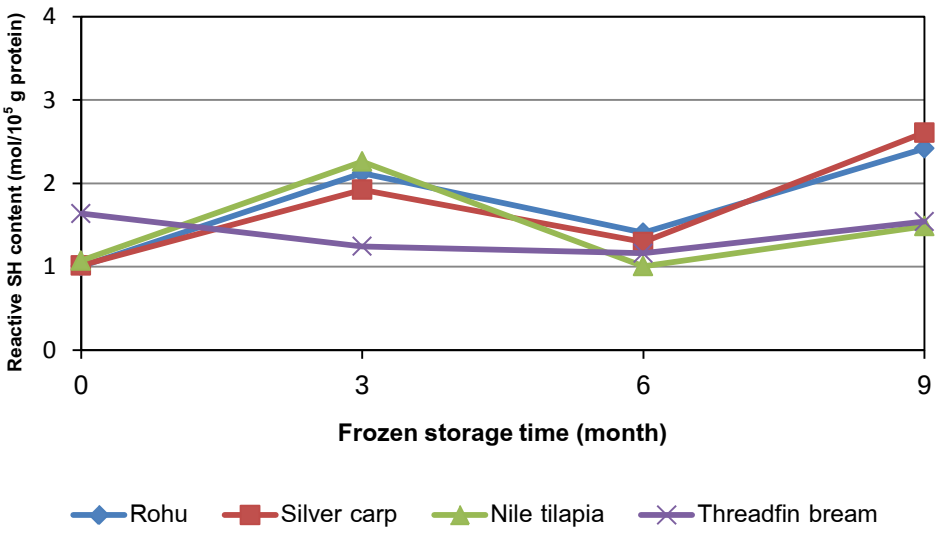

Figure3. Changes in reactive sulfhydryl content of surimi from four fish species during frozen storage at -20°C for 9 months**.**

3.4 Surface hydrophobicity

Changes in surface hydrophobicity were observed throughout the storage for up to 9 months (Fig. 4). In general, surface hydrophobicity of surimi from silver carp, rohu, nile tilapia increased during frozen storage. For threadfin bream surimi, it showed a higher initial surface hydrophobicity than other species. Its surface hydrophobicity gradually decreased during 9-months storage. The exposure of hydrophobic residues increased with frozen storage of freshwater species. However, hydrophobicity interactions of salt-extractable proteins appeared to increase during frozen storage of threadfin bream surimi. The results suggested the differences in frozen stability between freshwater and marine fish.

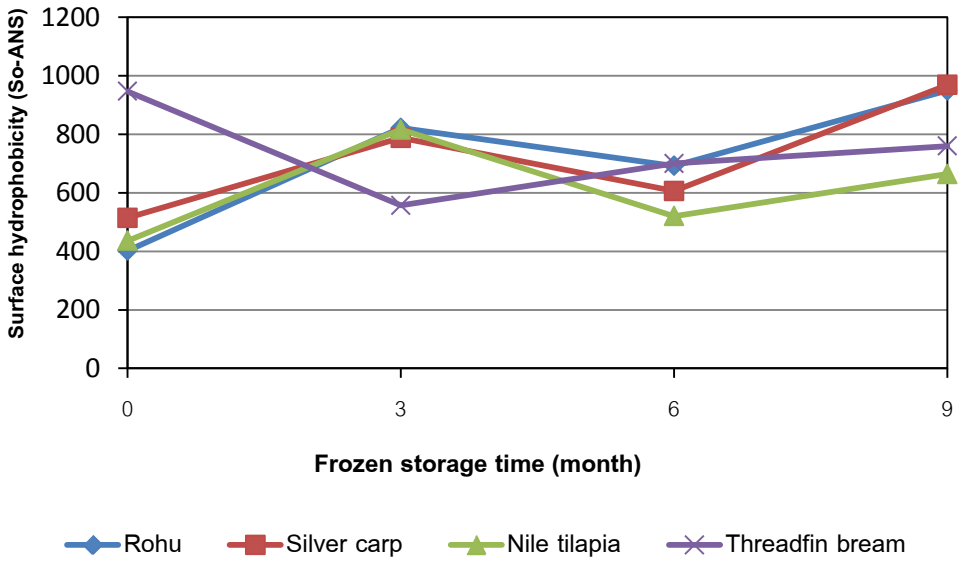

Figure 4. Changes in surface hydrophobicity of surimi from four fish species during frozen storage at -20°C for 9 months.

3.5 Textural properties of gel during storage

Breaking force and deformation of surimi from four fish species decreased throughout 9 months of frozen storage (Fig. 5A and 5B). Breaking force and deformation of rohu surimi decreased to the highest extent of 32 and 21%, respectively. After 9 months of storage at -20°C, breaking force of surimi from silver carp, nile tilapia and threadfin bream decreased by 30, 9 and 9%, respectively. Deformation of surimi from silver carp, nile tilapia and threadfin bream were decreased by 20, 1 and 2%, respectively. This result suggested that frozen protein denaturation was induced by extended frozen storage. The protein of rohu muscle was protein the most sensitive to frozen denaturation, resulting in the highest loss of gel-forming ability. The continuous decrease in both breaking force and deformation still occurred as storage time increased up to 9 months. This was presumed to be due to the increased denaturation of protein, which was induced by extended frozen storage as shown by the increase in reactive sulfhydryl group and the loss in protein solubility. Based on this study, surimi from freshwater fish was more prone to loss in gel forming-ability compared to threadfin bream. MacDonald et al. (1992) found that gel-forming ability of hoki muscle also decreased with the frozen storage time.

A decrease in gel-forming ability of fish muscles during frozen storage was associated with the frozen denaturation of surimi actomyosin via the aggregation of protein chains, leaving them unavailable for subsequent gel formation. Denaturation of myosin during frozen storage resulted in

an inferior gel network formation, causing a lower elasticity with poor water-holding capacity in the gel matrix (Benjakul et al., 2003). Thus, frozen storage directly affected the conformational changes in protein molecules, leading to the loss in functionality as observed by the decrease in gel-forming ability.

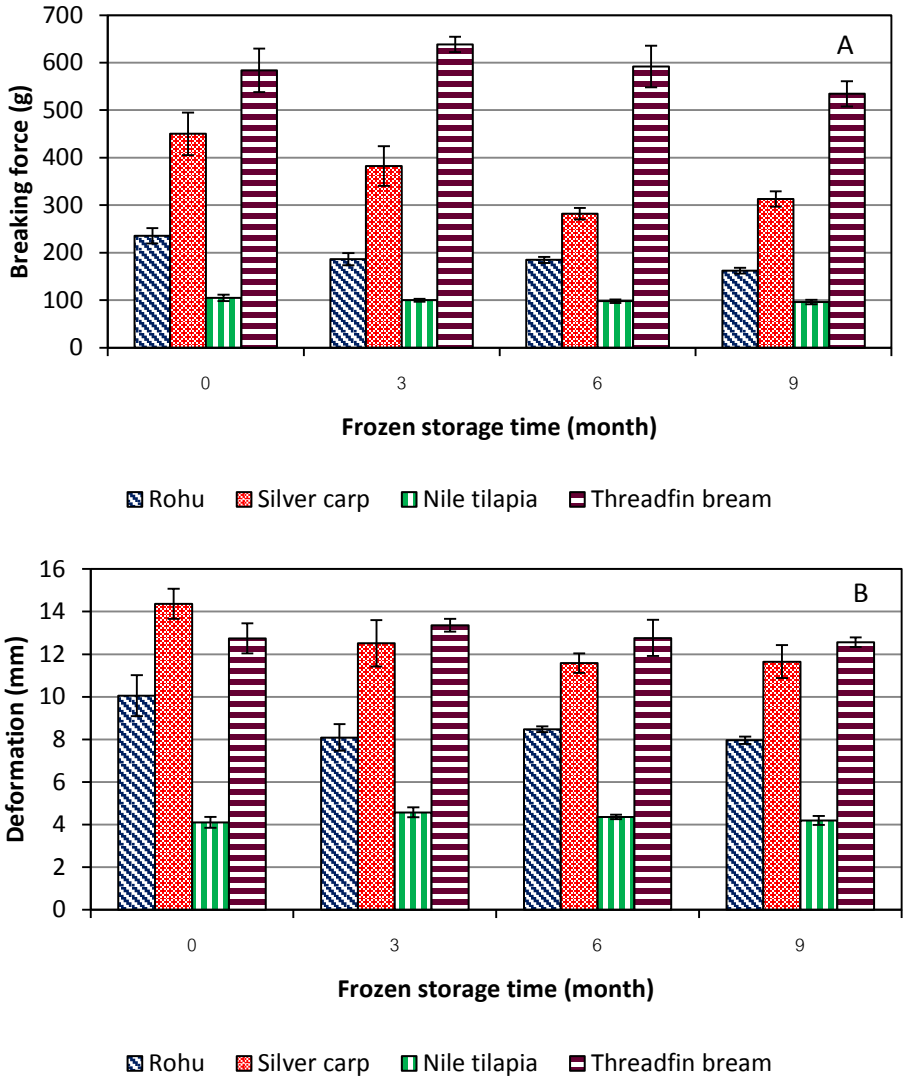

Figure 5. Changes in breaking force (A) and deformation (B) of surimi gels prepared from four fish species during frozen storage at -20°C for 9 months.

3.6 Whiteness

No marked changes were observed in whiteness of surimi gels from all species during 9-months of frozen storage (Table 1). Pigments could be removed effectively by washing process.

Threadfin bream rendered the lowest whiteness compared to the others. Differences in whiteness were possibly caused by variations in pigment content in each fish species.

Table 1. Whiteness of surimi gel prepared from four fish species during frozen storage at -20°C for 9 months.

Storage time (month)	Rohu	Siver carp^{ns}	Nile tilapia	Threadfin bream^{ns}
0	70.96±0.75^{a}	75.70±0.85	74.44±0.42^{b}	65.31±0.45
3	71.54±0.19^{a}	75.46±0.27	74.82±0.21^{b}	66.21±0.56
6	69.96±1.02^{b}	74.98±0.29	76.12±0.52^{a}	65.56±0.70
9	70.58±0.32^{ab}	75.08±0.81	75.12±0.14^{b}	65.34±0.48

Values in the same colume with different letters mean significant difference ($p<0.05$)

ns : no significant difference ($p>0.05$).

4. Conclusions

Extended frozen storage of four fish species at – 20°C caused the loss in gel-forming ability, which was associated with protein denaturation. Degree of changes was dependent upon species and storage time. Silver carp surimi maintained the highest gel-forming ability as compared to other freshwater species studied. Silver carp formed good gel but all freshwater surimi showed less frozen stability than threadfin bream surimi.

Acknowledgements

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References

Asgharzadeh, A., Shabanpour, B., Aubourgb, S. P. and Hosseinic, H. (2010). Chemical changes in silver carp (*Hypophthalmichthys molitrix*) minced muscle during frozen storage: Effect of a previous washing process. 61(1): 95-101.

Benjakul, S., Visessanguan, W., Thongkaew, C. and Tanaka, M. (2003). Comparative study on physicochemical changes of muscle protein from some tropical fish during frozen storage. Food Research International. 36(8): 787-795.

Benjakul, S., Visessanguan, W., Thongkaew, C. and Tanaka, M. (2005). Effect of frozen storage on chemical and gel-forming properties of fish commonly used for surimi production in Thailand. Food Hydrocolloids. 19: 197-207.

Bradford, M. (1976). A rapid sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 72, 248–252.

Chan, J. K., Gill, T. A., Thompson, J. W. and Singer, D. S. (1995). Herring surimi during low temperature setting, physicochemical and textural properties. Journal of Food Science. 60(6): 1248–1253.

Chomnawang, C., Nantachai, K., Yongsawatdigul, J., Thawornchinsombut, S. and Tungkawachara, S. (2007). Chemical and biochemical change of hybrid catfith fillet stored at 4°C and its gel properties. Food Chemistry. 103: 420-427.

Hayakawa, S., and Nakai, S. (1985). Relationships of hydrophobicity and net charge to 12 solubility of milk and soy proteins. Journal of Food Science. 50: 486–491.

MacDonald, G. A., Lelievre, J. and Wilson, N. D. C. (1992). Effect of frozen storage on the gel-forming properties of hoki (*Macruronus novaezelandiae*). Journal of Food Science. 57: 69–73.

Park, J.W., Yongsawatdigul, J., and Lin, T.M. (1994). Rheological behavior and potential cross-linking of Pacific whiting (*Merluccius productus*) surimi gel. Journal of Food Science. 59: 773-776.

Roura, S. I. and Crupkin, M. (1995). Biochemical and functional properties of myofibrils from pre and post-spawned hake (*Merluccius hubbsi Marini*) stored on ice. Journal of Food Science. 60: 269–272.

Yongsawatdigul, J. and Park, J. W. (2002). Biochemical and conformational changes of actomyosin from threadfin bream stored in ice. Journal of Food Science. 67(3): 985–990.

Yongsawatdigul, J. and Park, J.W. (2003). Thermal denaturation and aggregation of Threadfin bream actomyosin. Food Chemistry. 83: 409-416.

Zhou, A., Benjakul, S., Pan, K., Gong, J. and Liu, X. (2006). Cryoprotective effects of trehalose and sodium lactate on tilapia (*Sarotherodon nilotica*) surimi during frozen storage. Food Chemistry. 96(1): 96-103.