

EFFECT OF CRYOPROTECTANTS ON THE QUALITY OF SURIMI DURING STORAGE AT -20°C

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Abstract

Milkfish (Chanos chanos) is one of the abundant white-fleshed fish species caught in both Indonesia and Taiwan. This fish have good white color contents and gel elasticity contributing in making quality surimi. Surimi is an intermediate product containing stabilized myofibrillar proteins obtained from mechanically deboned fish flesh washed with water to remove sarcoplasmic protein, blended with cryoprotectants. The aim of this research is to study cryoprotectants effect on fish protein. Milkfish was bought from traditional fish markets and quickly transported to laboratory to avoid fish deterioration, fish were washed and cleaned to remove sewage and remain blood and then, it was weighed and discarded the head, fins, tail, scales, and guts, etc., then filleted and de-boned to take meat off from fish. The result showed Mixing of 4% sucrose and 0.2% chitosan for surimi could maintain its quality after 3 months storage, from results of VBN, gel strength, Ca^{2+} -ATPase activity. However, water holding capacity decline, and color of kamaboko had no significant difference than other treatment groups. Comparing to control group, the Ca^{2+} -ATPase activity and gel strength of each group milkfish surimi are higher ($p < 0.05$) than those of control within the storage period, however all cryoprotectants treated groups are not significant different ($p > 0.05$) each other. From this studies, it can be concluded that the longer storage the lower quality of surimi and the presence of cryoprotectants have important role to retain milkfish muscle during 3 months of frozen storage. Besides, addition of cryoprotectants can restrain the denature rate of myofibril protein.

Keywords: chitosan, sucrose, surimi, kamaboko, frozen storage

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1. INTRODUCTION

Surimi is an important intermediate product containing stabilized myofibrillar proteins obtained from mechanically deboned fish flesh that is washed with water to remove sarcoplasmic protein, blended with cryoprotectants and then used in the production of fabricated seafood products (Dora and Dey, 2010). It possesses some important functional properties such as gel-forming ability and water-holding capacity. Therefore, it has become the intermediate material for surimi-based products. Surimi can be produced from both marine and fresh water fish. Generally the type of fish used for surimi from species that are less economical/underused and from the fleshy white fish species, because the fleshy white fish have a high elasticity.

Chitosan has been used to maintain the functional properties of surimi. Besides, other

cryoprotectant (sucrose) is also used for surimi to prevent protein denaturation. However few attempts have focused on the effects of adding different concentration of cryoprotectants on the quality of surimi.

Chitosan is a low acetyl substituted form of chitin, composed of glucosamine, 2 amino- 2-deoxy-glucose (Shahidi et al., 1999), which has been used as an active material for its functional properties like antimicrobial (Cuero, 1999), texturizing (Benjakul et al., 2001), binding (No et al., 2000), emulsifying (Cho et al., 1998) and antioxidant activities (Kamil et al., 2002).

Chitosan can improve the gel strength of surimi which is considered as single most important parameter for quality and price (Kataoka et al., 1998). The strength of gels was nearly doubled by the addition of 1.5% chitosan when salted surimi pastes were set below 25°C (Kataoka et al., 1998). Chitosan

can enhance rheological properties in surimi with poor gel forming capacity, depending on the type and concentration of the chitosan used and on the system to which it is added (Benjakul et al., 2001; 2003; Jo et al., 2001; Lin and Chao, 2001).

The aim of this this research study the influence of different concentration of cryoprotectants on the quality milkfish surimi during frozen storage.

2. MATERIAL AND METHODS

Raw materials

Milkfish (*Chanos chanos*) were purchased from fish sellers at Bisha fish market, Keelung, Taiwan. The fishes were transported to the laboratory immediately by icing condition. Fishes were processed approximately within 24–36 hours after harvest.

Surimi preparation

On arrival at the Laboratory, fishes were washed and cleaned to remove sewage and remain blood and then, it was weighed and discarded the head, fins, tail, scales, and guts, etc., then filleted and de-boned to take meat off from fish. Minced muscle was mixed with water at a ratio 1:3 (w/w) and stirred for 10 min. Then held without stirring for 5 min at 10°C. Excess water was removed with a hand press. Various concentration of chitosan and sucrose (control, 0.2% chitosan solution, 1% chitosan, 4% sucrose, 4% sucrose + 0.2% chitosan and 4% sucrose + 1% chitosan) as treatments was prepared and add into surimi. The 0.2% chitosan solution was made by dissolved 3g chitosan powder with 1% acetic acid. Surimi was mixed with different kind and concentration of cryoprotectants (treatments) using a Hobart mixer. Surimi without cryoprotectant is used as control. Surimi was packed into polyethylene bags (150g each one) and to be slow frozen during 5 h at -20°C and then stored same condition and taken for analysis (preparation of natural actomyosin, measurement of ATPase, volatile base nitrogen, and pH at 0, 2, 30, 60, and 90 days. Each of experiment was carried out 3 replicates. Then, the remain surimi frozen surimi at 0, 2, 30, 60,

and 90 days storage was be thawed to prepare kamaboko.

Preparation of natural actomyosin and measurement of CA^{2+} -ATPase activity natural actomyosin (NAM)

Take 5 surimi and homogenate with 9 times volume of chilled (4°C) Webber's buffer (0.6 KCl), then it is centrifuged at 4°C (10,000 xg) 20 min. Supernatant is added 2 times volume of chilled (4°C) de-ion water and shaken, after that at 4°C (10,000 xg) 20 min. The precipitant is added same volume of chilled (4°C) Webber's buffer (1.2 KCl) and stirred for 30 min in cold condition, then then it is centrifuged at 4°C (10,000 xg) 20 min. The supernatant is make sure 100-200 mL chilled (4°C) Webber's buffer (0.6 KCl).

Test group (1.0 ml NAM) and control group (1.0 ml of 0.6 KCl buffer solution) are added with 0.50 M tris maleate pH 7.0 (0.50 ml), 0.10 M $CaCl_2$ (0.50 ml), H_2O (5.30 ml) and stored in ice box. The control group is mixed well with 5.0 ml of 15 trichloroacetic acid at first. After put into incubator 25°C water bath, each tube was added 0.5 ml substrate of 20 M ATP (pH=7.0) solution and exactly 5 min incubation when it is warm up. After exactly 5 min incubated at 25°C water bath, each one of test group is added 5.0 ml (15%TCA) to stop reaction. After filtrate with no. 5 filtrate paper, take 1.0 ml of the filtrate inti tube and then added 2.5 de-ion water, 0.5 ml Elon solution and 1.0 ml of ammonium molybdate sulfate solution. After 45 min color formation, it is detected the absorbance at UV 640 nm.

Volatile basic nitrogen

Initially sample weighed 5 grams, and added 50 ml of TCA solution and homogenized for 1 minute. Homogenization results in the filtered solution with filter paper so that the filtrate is filtered clear. 1 ml solution of boric acid incorporated into the inner chamber cup Conway. Using the size of another pipette 1 ml of the filtrate to enter the outer chamber in the right, so it has not been mixed with the filtrate. Conway bowls immediately sealed with vaseline in the previous spread. Besides doing well in the blank where the filtrate sample exchange with a solution of 5% TCA and

treated as the same procedure. The Cup of Conway on rocking for 1 minute, then incubated at 35 degrees C for 2 hours. later in the titration with 0.02 N HCl solution into the sample (a) and blank (b) until the color of the solution to pink.

calculation:

$$\text{VBN (mg/100 gr)} = \{[0.28 \times (a - b) \times f \times 50]/5\} \times 100$$

f = factor of 0.02 N HCl solution calibration

Kamaboko preparation

Kamaboko gels were prepared from each surimi samples according to the method of Ian et al. (1995) with slightly modified. The frozen surimi samples were thawed at 4°C for 2 hours by putting in ice box. Each of one hundred gr surimi with different treatments were prepared and 3% NaCl and 5% starch were sprinkled on to the surimi, and the fish-salt-starch mixture was ground at 5°C for 10 min. The surimi paste was then stuffed into bag cloths and were sealed tightly using polyvinylchloride film and rubber bands. Two-step heated gels were carried out by setting at 40°C for 1 h, followed by heating at 90°C for 30 min in a water bath. After cooking, gels are immersed in ice bath, and then stored in a cold room for later evaluation of color, gel strength, and fold test.

Texture analysis (gel strength)

The texture of surimi gel was measured by a texture analyser (Model TA-XT2, Stable MicroSystems, Godalming, Surrey, England). Gels were equilibrated and tested at room temperature. Five cylinder-shaped samples of 2.5 cm in length were prepared from each gel. The breaking force (gel strength) and deformation (elasticity/ cohesiveness) were measured by using the texture analyser equipped with a cylindrical plunger (5 mm diameter; 60 mm⁻¹ penetration speed) (Benjakul et al., 2001).

Folding test

A surimi gel slice (3 cm diameter and 3 mm thick) was folded by thumb and orefinger. Five stages were used to evaluate the sample (Kudo et al., 1973):

AA (5)=no cracks showing after folding twice, A(4)=no cracks showing after folding in half, B(3)=cracks gradually when folded in half, C

(2)=cracks immediately when folded in half, and D(1)=breaks by finger pressure.

Water holding capacity (WHC)

WHC of untreated and treated fillets was determined by the method of Himonides et al. (1999) with slightly modified. For each treatment, three samples (each of initial weight 2.00 ± 0.50 g) were wrapped in individual Whatman filter papers (No. 41, with an Ø of 7.0 cm) and centrifuged (Beckman, GS-15R centrifuge) at 3500 rpm for 30 min at room temperature. The quantity of water expelled from the fish tissue was estimated from the weight difference of the filter paper before and after centrifugation. The WHC of the fish tissue was determined by the following equation (Himonides et al., 1999):

$$\text{WHC (\%)} = (\text{Mw}/\text{Ms}) \times 100$$

Where Mw is the mass (g) of expelled water and Ms is the initial mass (g) of the sample.

Experimental design and statistical analysis

This experiment consists of two factors. The first factor was the concentration cryoprotectants, consisting of six levels, namely 0% chitosan, 0.2% chitosan 1% chitosan, 4% sucrose, 4% sucrose + 0.2% chitosan, and 4% sucrose + 1% chitosan of the weight of surimi. The second factor was prolong frozen storage of surimi, consists of five level (0, 2, 30, 60, and 90 days). The combination treatment in this study is 6 x 5 = 30 (thirty) combined treatment. All analyses are run in triplicate for each treatments. Analysis of variance was performed by ANOVA procedures (SPSS 19.0 for Windows, SPSS Inc., Chicago, IL). Differences among the mean values of the factors of treatments are determined by the Tukey test, and the significance was defined at $p < 0.05$. The data gain after experiment are computed and draw figure by using Excel software.

3. RESULTS AND DISCUSSION

The results are summarized in Figure 1. The figure shows the prolong of frozen storage increased the value of volatile base nitrogen, however adding cryoprotectant could inhibit the increase of VBN slowly. At 0 day storage, VBN value of the control, 0.2% chitosan, 1%

chitosan, 4% sucrose, 4% sucrose+0.2% chitosan, 4% sucrose+1% chitosan, are 6.92, 6.52, 7.32, 7.18, 6.65 and 5.72 mg/100 g, respectively. TVB-N at -20°C for 90 days in the control rise to 14.14 mg/100g. However, treatment group adding cryoprotectants 0.2% chitosan, 1% chitosan, 4% sucrose, 4% sucrose + 0.2% chitosan, 4% sucrose+1% chitosan reach to 8.65, 9.09, 9.93, 8.82, and 8.96 mg/100g, respectively. **Volatile basic nitrogen (VBN)**

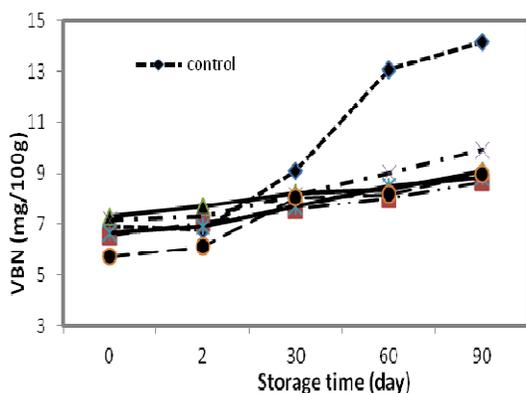


Figure 1. The volatile basic nitrogen of milkfish surimi with various cryoprotectants during storage at -20°C

Comparing the various cryoprotectants, milkfish surimi comprising 0.2% chitosan exhibit the lower VBN value ($p < 0.05$) than that of other cryoprotectants, but there was a slightly difference as 0.2% chitosan+4% sucrose (Figure 1). The presence of cryoprotectants can be delayed the declined quality of milkfish surimi.

Chitosan is a natural biopolymer that must be dissolved in a slightly acidic solution in order to activate its antimicrobial activity and inhibit the growth of microorganism. The reason for antimicrobial action of chitosan may be due to its ability to absorb nutrients of bacteria and thus inhibition of their growth (Knorr, 1991). From the result, the use of 0.2% chitosan was suitable to inhibit the growth of microbe after storage 90 days. Our studies shows at 90 day storage periode, VBN contents by addition 0.2% chitosan is significant different ($p < 0.05$) than control, 1% chitosan, and 4% sucrose, but not significant different than 4% sucrose + 0.2% chitosan and 4% sucrose and 1% chitosan.

Ca²⁺ATPase activity

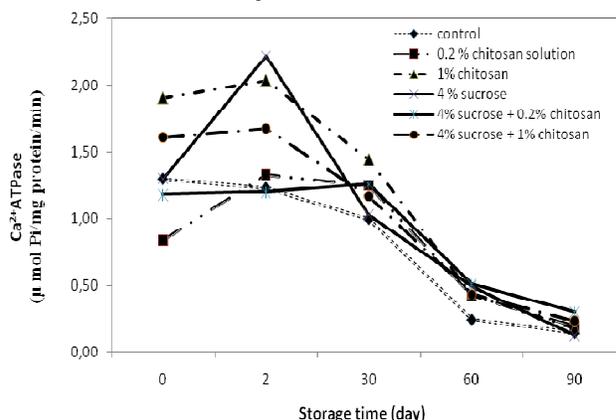


Figure 2. Ca²⁺ATPase activity of milkfish surimi with various cryoprotectants during storage at -20°C

The effect of different cryoprotectants and temperature Ca²⁺-ATPase activity are illustrated Figure 2. Our result show that addition of cryoprotectants can maintain Ca²⁺-ATPase activity during frozen. The Ca²⁺-ATPase contents after 90 days frozen storage for the control, 0.2% chitosan, 1% chitosan, 4% sucrose, 4% sucrose+0.2% chitosan, 4% sucrose+1% chitosan dropped to 0.145, 0.197, 0.174, 0.124, 0.301, and 0.232 µ mol Pi/mg protein/min, respectively. It seems that Ca²⁺-ATPase activity declines during frozen storage, but cryoprotectants are able to maintain Ca²⁺-ATPase activity. From the result, the decline of ATPase is due to myosin denaturation during extended frozen storage. The treatment by adding 4% sucrose + 0.2% chitosan has the highest Ca²⁺-ATPase activity ($p < 0.05$).

Benjakul and Bauer (2000) explain that the loss in ATPase activity was due to tertiary structural changes caused by ice crystals and an increase in the ionic strength of the system. They also assumed that rearrangement of proteins via protein-protein interactions contributed to the loss in ATPase activity. Hashimoto et al. (1982) and Hashimoto et al (1985), have conducted research in walleye pollack surimi. Their result showed that myosin of walleye pollack is quite thermally unstable. Myofibrillar proteins in surimi made from this species are kept stable by adding cryoprotectants at effective concentrations. The result from this research,

the decline in the Ca^{2+} -ATPase activity of raw surimi indicates the large degree of denaturation of myosin. The added cryoprotectants, however, are able to provide protection against drastically reduce Ca^{2+} -ATPase activity. According to this experiment, surimi treated with a cryoprotectant can maintain its Ca^{2+} -ATPase activity during frozen storage. This study also prove that combination both between 0.2% chitosan solution and 4% sucrose were slightly better at maintaining Ca^{2+} -ATPase activity than adding either sucrose or chitosan alone during 3 months of frozen storage. From our studies, it can be concluded that treatment by adding 4% sucrose + 0.2% chitosan is significant different ($p < 0.05$) within 90 day storage at -20°C .

Water holding capacity (WHC)

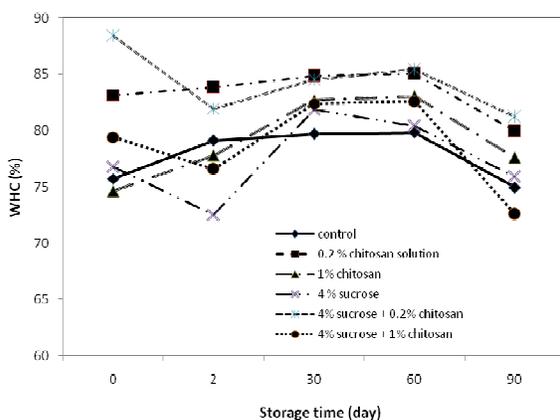


Figure 3. WHC changes kamaboko made from milkfish surimi with various cryoprotectants during storage at -20°C

Figure 3. demonstrates the effect of storage and different cryoprotectant on WHC. At 0 day, the initial contents of WHC for the control, 0.2% chitosan, 1% chitosan, 4% sucrose, 4% sucrose+0.2% chitosan, 4% sucrose+1% chitosan, are 75.73, 83.17, 74.63, 76.81, 81.95, and 79.41%, respectively. Besides, it can be seen that the WHC contents after 30 days frozen storage for the control, 0.2% chitosan, 1% chitosan, 4% sucrose, 4% sucrose+0.2% chitosan, 4% sucrose+1% chitosan rises to 79.69, 84.84, 82.71, 81.82, 88.41, and 82.41%, respectively. On the other hand, at 90 day,

WHC kamaboko go down to 74.96, 79.97, 77.60, 75.89, 81.25 and 72.59, respectively.

According to Soeparno (1992), water holding capacity is also affected by the freshness of the fish are in a state of prerigor or postrigor, as more and more fresh (prerigor) fish, the higher the water holding capacity. Aberle et al. (2001) added that one of the most factor affecting WHC is protein content which the protein molecules bind the water molecule. Moreover, Dey and Dora (2010) mentioned that chitosan could effectively increase the water holding capacity of muscle protein. The study from Velazquez et al. (2007) showed the increase in the surimi pastes WHC caused by adding the Chitosan-Alginate soluble protein mixture into the fish paste caused a decrease in firmness and consistency.

Gel strength

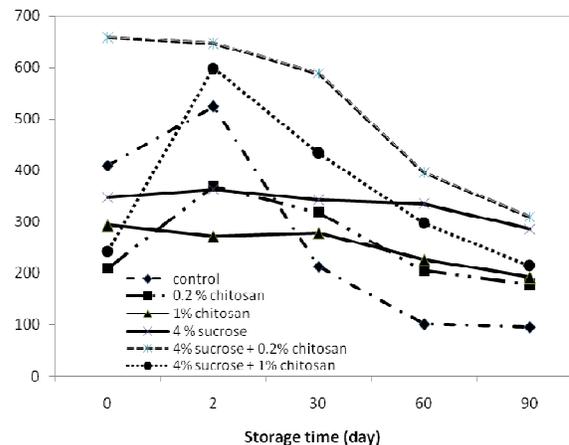


Figure 4. The gel strength of milkfish surimi with various cryoprotectants during storage at -20°C

This experiment shows that 4% sucrose blended with 0.2% chitosan treatment group has the highest value of the variables WHC after 90 day storage time ($p < 0.05$). From our studies, 4% sucrose + 0.2% chitosan is significant different ($p < 0.05$) than others treatments, except 0.02% chitosan not significant different ($p > 0.05$).

The gel strength of surimi from milkfish are displayed in Figure 4. The lowest of gel strength is from control (96.010 g.cm), after storage time during 90 day, the highest gel strength is from 4% sucrose blended with 0.2%

chitosan (309.354g.cm). The longer frozen storage decline the gel strength of milkfish surimi, the presence of cryoprotectant can retain the gel strength of milkfish surimi not to be dropped drastically. From the result, it can be concluded that 4% sucrose blended with 0.2% chitosan is the best compare with other cryoprotectants and can maintain the gel strength of milkfish surimi during frozen storage and gave the higher gel strength value than that other cryoprotectants. The value of gel strength decline coincide with prolong storage. The decrease of gel strength surimi is possibly the protein degradation occurred.

Kataoka *et al.* (1997) carried out the research by the addition of chitosan or chitin. From their experiment, they summarized that the free amino groups of chitosan play an important role in producing a strong gel from low quality walleye pollock surimi. Kok (2005) have conducted experiment, from his result the addition of acid at the level used in the study might not cause the whole myofibril protein to swell and dissolve, but might cause partial solubilization of the outer layer of the myofiber or myofibrils. During gelation, solubilized proteins crosslink with other myofibrils, thus making the gel firmer. In addition, studies carried out by Naftalian and Symons (1974) indicated that the stabilization of sugars is a structural effect and sucrose gives higher levels of stabilization. They suggest that the stabilization is due to hydrogen bonding properties. Finch *et al.* (1974) observed that an increase in sucrose content in the gel solution resulted in a slight increase in gel strength. It can be presumed that sugars are found to increase and cooperate the strength of chitosan gels.

Folding test

Folding test kamaboko from milkfish during storage by adding different various cryoprotectants are displayed in Table 1. At 0 day, initial folding test score of kamaboko with conditions control, 0.2% chitosan solution, 1% chitosan, 4% sucrose, 4% sucrose + 0.2 % chitosan, 4% sucrose + 1% chitosan are A, A, A, AA (which means elatisitas surimi gel products is very good and has the ability to be

folded into a quarter circle), and AA. B symbolizes cracks immediately when folded in half, AA means no cracks showing after folding twice, and A is no crack showing after folding in a half. From the 0 days show that 4% sucrose combined with 0.2% chitosan and 4% sucrose mixed with 1% chitosan have the higher folding test than those other cryoprotectants.

Table 1. Folding test of kamaboko made from milkfish adding with various cryoprotectants during frozen storage

Analysis	Treatments	Storage Time (Days)				
		0	2	30	60	90
Folding test	control	A	B	B	C	C
	0.2 % chitosan solution	A	A	A	A	B
	1% chitosan	AA	A	A	A	B
	4 % sucrose	A	A	A	A	B
	4% sucrose + 0.2% chitosan	AA	A	A	A	A
	4% sucrose + 1% chitosan	AA	A	A	A	B

After 90 days storage time, 4% sucrose blended with 0.2% chitosan give the better folding test on kamaboko than those of other cryoprotectants. The table shows, there is no change on the folding test score kamaboko from milkfish. It can be concluded that those cryoprotectants is the best in this experiment.

4. CONCLUSIONS

The aim of this research is to investigate the effects of fish freshness on kamaboko quality and to study the influence of different concentration of cryoprotectants on the quality milkfish surimi during frozen storage. The decrease of Ca^{2+} -ATPase of surimi was concomitant with the decrease of gel strength, and very related each other and mixing 4% sucrose with 0.2% chitosan gave the better value on Ca^{2+} -ATPase activity, gel strength and folding test than other treatments whereas 0.2% chitosan gave good analysis on volatile base nitrogen on surimi quality. Overall, comparing with adding sucrose or chitosan alone, the mixing of two those cryoprotectants gave optimum effect on surimi quality

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6. REFERENCES

- [1] ABERLE, E.D., FORREST, J.C., GERRAND, D.E. MILLS, E.W. 2001. Principles of meat science, 4th ed. Kendall Hunt Publishing Company, Dubuque, Iowa.
- [2] Auh, J.H., Lee, H.G. J. W. Kim, J.W., Kim, J.C., Yoon, H.S. Park, K.H. 1999. Highly Concentrated Branched Oligosaccharides as Cryoprotectant for Surimi. Journal Of Food Science, 64: 418-422.
- [3] BENJAKUL, S., VISESSANGUAN, W., TANAKA, M., ISHIZAKI, S., SUTHIDHAM, R. SUNGPECH, O. 2000. Effect of chitin and chitosan on gelling properties of surimi from barred garfish (*Hemiramphus far*). Journal of the Science of Food and Agriculture, 8, 102-108.
- [4] BENJAKUL, S., VISESSANGUAN, W. SRIVILAI, C. 2001. Porcine plasma proteins as gel enhancer in bigeye snapper (*Priacanthus tayenus*) surimi. Journal of Food Biochemistry, 25, 285-305.
- [5] BENJAKUL, S. BAUER, S. 2000. Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze-thaw cycles. Journal of Food Science Agriculture, 80, 1143-1150.
- [6] BENJAKUL, S., VISESSANGUAN, W., PHATCHRAT, S. TANAKA, M. 2003. Chitosan affects transglutaminase induce surimi gelation. Journal of Food Biochemistry, 27, 53-66.
- [7] CHO Y.L., NO H.K., MEYERS S.P. 1998. Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. Journal Agriculture Food Chemistry, 46, 3839-3843.
- [8] CUERO, R.G. 1999. Antimicrobial action of exogenous chitosan. Birkhauser Verlag Basel, Switzerland.
- [9] DEY, S.S., DORA, K.C. 2010. Suitability of chitosan as cryoprotectant on croaker fish (*Johnius gangeticus*) surimi during frozen storage. Journal of Food Science and Technology, 6, 699-705.
- [10] FINCH A., GARDNER P.J., LEDWARD D.A., MENASHI S. 1974. The thermal denaturation of collagen fibers swollen in aqueous solution of urea, hexamethylenetetramine, P- benoquinone andtetraalkylammonium salts. Biochemica Biophysica Acta, 365, 400-7.
- [11] HASHIMOTO A, KOBAYASHI A, ARAI K. 1982. Thermostability of fish myofibrillar Ca-ATPase and adaptation to environmental temperature. Nippon Suisan Gakkaishi, 48: 671-684.
- [12] HASHIMOTO A, KATOH N, NOZAKI, H. ARAI K. 1985. Effect of storage temperature on deterioration of salted meat paste from three fish species. Nippon Suisan Gakkaishi, 51, 847-853.
- [13] JO, C., LEE, J.W., LEE, K.H., AND BYUNA, M.W. 2001. Quality properties of pork sausage prepared with water-soluble chitosan oligomer. Meat Science, 59, 369-375.
- [14] KAMIL, J., SHAIDI, F., JEON, Y.J., KIM, S.K. 2002. Antioxidant role of chitosan in a cooked cod (*Gadus morhua*) model system. Journal of Food Lipids, 9, 57-64.
- [15] KATAOKA, J., ISHIZAKI, S. TANAKA, M. 1997. Effects of chitosan on gelling properties of low quality surimi. Journal of Muscle Foods, 9, 209-220.
- [16] KNORR. D. 1991. Recovery and utilization of chitin andchitosan in food processing waste management, Food Technology, 45: 114-122.
- [17] KOK, T.N. 2005. Biochemical and Physical Factors Affecting Fish Ball. Thesis Master of Science in Food Science and Technology, Oregon State University.
- [18] LIM, S.T., LEE Y.S YOO, B. 2005. Effect of sugars on thermal gelation of surimi sols. Food Science and Biotechnology, 14, 340-343.
- [19] LIN, K.W. CHAO, J.Y. 2001. Quality characteristics of reduced-fat Chinese-style sausages as related to chitosan's molecular weight. Meat Science, 59, 343-351.
- [20] NAFTALIAN R.J., SYMONS, M.C.R. 1974. The mechanism of sugar dependent stabilization of gelatin gels. Biochimica Biophysica Acta, 352, 173-76.
- [21] NO, H. K., LEE, K, S., MEYERS. S. P. 2000. Correlation between physicochemical characteristics and binding capacities of chitosan products. Journal of Food Science, 65, 1134-1137.
- [22] NOPIANTI, R.,HUDA, N., FAZILAH, A.,ISMAIL, N., EASA, A. M. 2012. Effect of different types of low sweetness sugar on physicochemical properties of threadfin bream surimi (*Nemipterus spp.*) during frozen storage. International Food Research Journal, 19, 1011-1021.
- [23] PAN J., SHEN, H. AND LUO, Y., 2008. Cryoprotective effects of trehalose on grass Carp (ctenopharyngodon idellus) surimi during Frozen storage. Journal of Food Processing and Preservation, 34, 715-727.
- [24] SHAHIDI, F., ARACHCHI, J.K.V., YOU-JIN JEON. 1999. Food applications of chitin and chitosan. Trends in Food Science and Technology, 10, 37-51
- [25] SULTANBAWA, Y. LI-CHAN, E.C.Y. 1998. Cryoprotective effects of sugar and polyol blends in ling cod surimi during frozen storage. Food Research International, 31: 87-98.
- [26] SOEPARNO. 1992. Ilmu dan Teknologi Daging. Gajah Mada University, Yogyakarta.
- [27] WIBOWO, S., VELAZQUEZA, G., SAVANTA, V., TORRESA, J.A. 2007. Effect of chitosan type on protein and water recovery efficiency from surimi wash water treated with chitosan-alginate complexes. Bioresource Technology, 98, 539-545