

Effect of Formaldehyde on the Gel Forming Ability of Fish Meat

Orawan Kongpun¹, Pantip Suwansakornkul¹ and Patcharin Predalumpabut²

ABSTRACT

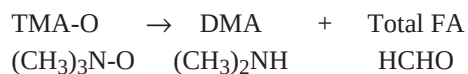
The gel forming ability of dorab meat and washed dorab meat with the addition of 0, 25, 50, 75, 100, 200, 300, and 500 ppm of formalin solution was determined. Three levels of heating: (1) 40°C 30 min, (2) 90°C 30 min, and (3) 40°C 30 min followed by 90°C 30 min were used for heating fish meat gel. The SDS-PAGE analysis was also carried out. The results showed that gel force, deformation and gel strength of dorab meat were higher than washed dorab meat with and without formalin addition at all heating temperatures ($P>0.05$). After the addition of formalin solution, the gel force, deformation and gel strength of fish meat gels decreased at all heating temperatures. The addition of formalin at ≥ 100 ppm affected the gel forming ability of washed dorab meat. Their gel strength was lower than 200 g.cm and formalin smell could be perceived. The fish gels heated at 40°C and 40°C/90°C provided higher gel properties than those heated at 90°C. According to a SDS-PAGE analysis, the protein patterns of samples without the formalin solution correlated to their gel forming ability. Samples with 100, 200, 300, 500 ppm formalin solution showed the loss of myosin heavy chain and actin bands at 90°C. It seemed likely that formaldehyde affected not only the gel forming ability of myofibrillar proteins but also the conformation of these proteins.

Key words : formaldehyde, gel forming ability, fish meat

INTRODUCTION

An important factor affecting the gel forming ability or quality of surimi is the freshness of fish (raw material). Generally, the chemical freshness index of the fish are the K-value, total volatile bases (TVB) and trimethylamine (TMA). Trimethylamine oxide (TMAO) has been naturally found in a large number of marine fish and shellfish. The reduction of TMAO to TMA, dimethylamine (DMA) and formaldehyde (FA) is caused by endogenous enzymes in fish and exogenous enzymes produced by bacteria during fish spoilage. However, several researchers reported that FA correlated to freshness of fish, especially lizard fish. It caused muscle protein denaturation and reduction of gel forming

ability (Nozaki *et al.*, 1978; Sophonphong and Rungjiratananan, 1993). Moreover, formation of DMA and FA by TMAO decomposition had taken place during storage at -5°C, -20°C and -25°C. (Nozaki *et al.*, 1978; Tunhun *et al.*, 1996). FA in fish meat can be classified as free form and combined form (Boeri *et al.*, 1993). The free form is easily extracted by trichloroacetic acid and determined by Nash's method. The FA combined with proteins (combined FA) could not be extracted. It is known that equal mole of DMA and FA is produced from TMAO (Owusu-Ansah and Hultin, 1986). Thus, the amount of combined FA can be calculated by the following equation.



¹ Fishery Technological Development Institute, Department of Fisheries, Chatuchak, Bangkok 10900 Thailand.

² Fish Inspection and Quality Control Division, Department of Fisheries, Songkla 90000, Thailand.

equivalent mole (mole DMA $\equiv$ mole total FA) (1)

Total FA = Free FA + Combined FA ... (2)

Therefore the objective of this experiment is to study the effect of formaldehyde, both free and combined forms, on the gel forming ability of fish meat at various heating temperatures.

MATERIALS AND METHOD

Whole fresh dorab (*Chirocentrus dorab*, Forskal), bought from Bangkok fish market organization, were sampled for chemical analysis. Proximate composition and pH were determined by the AOAC (1990) method. TVB and TMAO were estimated by Hasegawa (1992) methods. DMA was analysed by Dyer's method (Dyer and Mounsey, 1945) using copper ammonium reagent and carbondisulfide-toluene solution. FA was determined by Nash's method (Amano *et al.*, 1963) using acetylacetone reagent. The rest of the fish was headed, gutted and washed thoroughly in ice water. Fish meat was separated from bone and skin by a deboner machine. This minced meat was separated into 2 portions. The first one was mixed with 3% salt in a Stephan cutter-mixer for gel preparation. This fish meat gel was adjusted to 78% moisture. After mixing, the meat sol was filled into cellulose casings with a diameter of 3.5 cm and a length of 15 cm. Both ends of the casing were fastened tightly. The heating step was carried out at various temperatures of (1) 40°C 30 min, (2) 90°C 30 min, and (3) 40°C 30 min followed by 90°C 30 min. The samples were cooled in ice water and kept at 5°C for 18-24 h before gel evaluation.

The second portion of fish meat was washed in ice water 3 times (10 min each). The ratio of fish meat to ice water was 1 to 5 by weight. The fish meat was pressed by a hydraulic press to discard water and separated into 8 portions. Formalin solution (prepared from formaldehyde 40% w/v Carlo Erba, analysis reagent) was mixed with each portion at concentrations of 0, 25, 50, 75, 100, 200, 300, and

500 ppm, respectively. Each portion of fish meat was left at room temperature for 1 hour and processed for chemical analysis and gel preparation.

SDS-PAGE analysis of fish meat gel and surimi gel

Each gel of 0.5 g was solubilized with 20 ml of 0.05M sodium phosphate buffer (pH 8.0) containing 8M urea, 2%SDS and 10% 2-mercaptoethanol. SDS-PAGE was carried out on 5% polyacrylamide gel (vertical slab gel with 0.75 mm thickness) according to the method of Weber and Osborn (1969). The patterns of protein as polymers, myosin heavy chains (MHC) and MHC breakdown or degraded products were evaluated.

Gel forming ability (MFRD, 1988)

Gel force (g), deformation (mm) and a folding test of fish meat gel were determined. Samples of meat after gel preparation were cut into 2.5 cm thickness. Five pieces of gel were measured for gel force and deformation by a Rheometer with a spherical plunger (5 mm in diameter) at a speed of 60 mm/min. The sample was pressed by a plunger until a break occurred in the surface of sample. The weight exerted on the sample until its breaking point is called force and the depth of breaking point is called deformation.

Sample discs of 25 mm in diameter and 5 mm in thickness were folded and graded in a folding test according to the following scheme:

- AA No breakage when folded in quarters
- A Slight tear when folded in quarters
- B Slight tear when folded in half
- C Breakage (but 2 pieces still connected)

when folded in half

- D Complete breakage into 2 pieces when folded in half

RESULT AND DISCUSSION

The chemical analysis of unwashed and washed dorab meat are shown in Table 1. Protein,

fat, ash content and pH of fresh dorab meat were 17.62%, 1.97%, 1.24% and 6.5, respectively while moisture content was 77.20%. After washing, they changed to 15.21%, 1.34%, 0.38% and 6.8 while moisture was 81.20%. Total volatile bases (TVB) as freshness index was 6.86 mg/100g in unwashed dorab meat and decreased to 4.34 after washing. TMAO of dorab meat also decreased after washing from 13.39 mg/100g to 3.24 mg/100g. Yasui and Lim (1987) concluded that most of the TMAO in the minced meat was washed out, resulting in an increase of meat sol and salt soluble protein extraction.

Hiraoka *et al.* (1995) suggested that combined FA could play an important role in reducing the gel forming ability of fish muscle. The amount of total FA (estimated total FA), free FA and combined FA of fish sample are shown in Table 2. The amount of free FA of unwashed and washed dorab meat were 10.01 ppm and 13.55 ppm while total FA were 36.08 ppm and 38.43 ppm, respectively. Tunhun *et al.* (1996) found that free FA of chub mackerel, rake-gilled mackerel and Indo-Pacific king mackerel was below 1.0 mg/kg and that in silver pomfret was 1.5 mg/kg. After the addition of 0, 25, 50, 75, 100, 200, 300, and 500 ppm formalin solution, almost 80% of these solutions combined with fish meat while the rest was extracted and analysed as free FA. The obtained free FA was 13.55, 16.50, 17.13, 16.82, 18.93, 30.43, 61.92, and 81.94 ppm. Therefore, the combined FA of these

samples were 24.88, 46.25, 70.71, 95.91, 119.10, 206.10, 275.23 and 455.96 ppm, respectively (Table 2). Tunhun *et al.* (1996) also reported that the analysed concentration of free FA in some fresh fish correlated with the concentration of the dipping formalin solution.

The gel force of unwashed dorab meat was quite high as 532, 373 and 604 g when heated at 40°C 30 min, 90°C 30 min and 40°C 30 min followed by 90°C 30 min respectively. After washing, the gel force of washed dorab meat was lower than those of unwashed meat at all heating levels ($p < 0.05$). This result indicated that some gel-forming enhancing factor such as transglutaminase was removed during washing as also suggested by Seki *et al.*, 1990. The highest gel force of washed meat heated at 40°C 30 min followed by 90°C 30 min was 497 g while the lowest one heated at 90°C 30 min was 293 g. The higher concentration of formalin added, the lower gel force obtained. At low concentrations of 25 and 50 ppm, the gel force was almost the same as the non-added FA meat ($p > 0.05$) but decreased drastically when the higher concentrations of FA were added ($p < 0.05$). The decrease of gel deformation also observed in samples with high concentrations of FA. In addition, the gel strength of dorab meat gel with and without formalin added at all heating steps correlated to their gel force (Figure 1). The washed dorab meat of 25, 50, 75, 100 and 200 ppm of formalin added still had good gel forming ability at the heating level of 40°C 30

Table 1 Chemical analysis of dorab meat.

Sample	Protein (%)	Fat (%)	Moisture (%)	Ash (%)	pH	TVB (mg/100g)	TMAO (mg/100g)
Dorab meat	17.621/±0.262/	1.97±0.07	77.20±0.27	1.24±0.02	6.5	6.86±0.0	13.39±4.12
Washed dorab meat	15.21±0.06	1.34±0.07	81.20±0.33	0.38±0.03	6.8	4.34±0.13	3.24±0.22

1/ Mean of triplicate determination

2/ Standard deviation

min followed by 90°C 30 min. Their gel strength was higher than 100 g.cm and folding tests were AA. However, formalin smell could be perceived from samples with the addition of formalin from 100 to 500 ppm. Moreover, these samples heated at 90°C 30 min were rejected because of the detection of formalin smell and the low folding test as C. On the other hand the FA smell was detected when free FA and combined FA were >20 and 100 ppm, respectively. Samples with the addition of 200, 300 and 500 ppm formalin heated at 40°C for 30 min

were rejected by a folding test score of C while the formalin smell was also detected with the addition of formalin 100-500 ppm. According to Tunhun *et al.* (1996), a formaldehyde smell could be perceived in rake-gilled mackerel and lizard fish dipped in solutions containing more than 500 and 2,000 ml/L formaldehyde, respectively. These results indicated that the increase of FA content in fish meat of more than 20 ppm as free FA or 70 ppm as combined FA would affect the gel forming ability. It has been reported that the reaction of formaldehyde causes

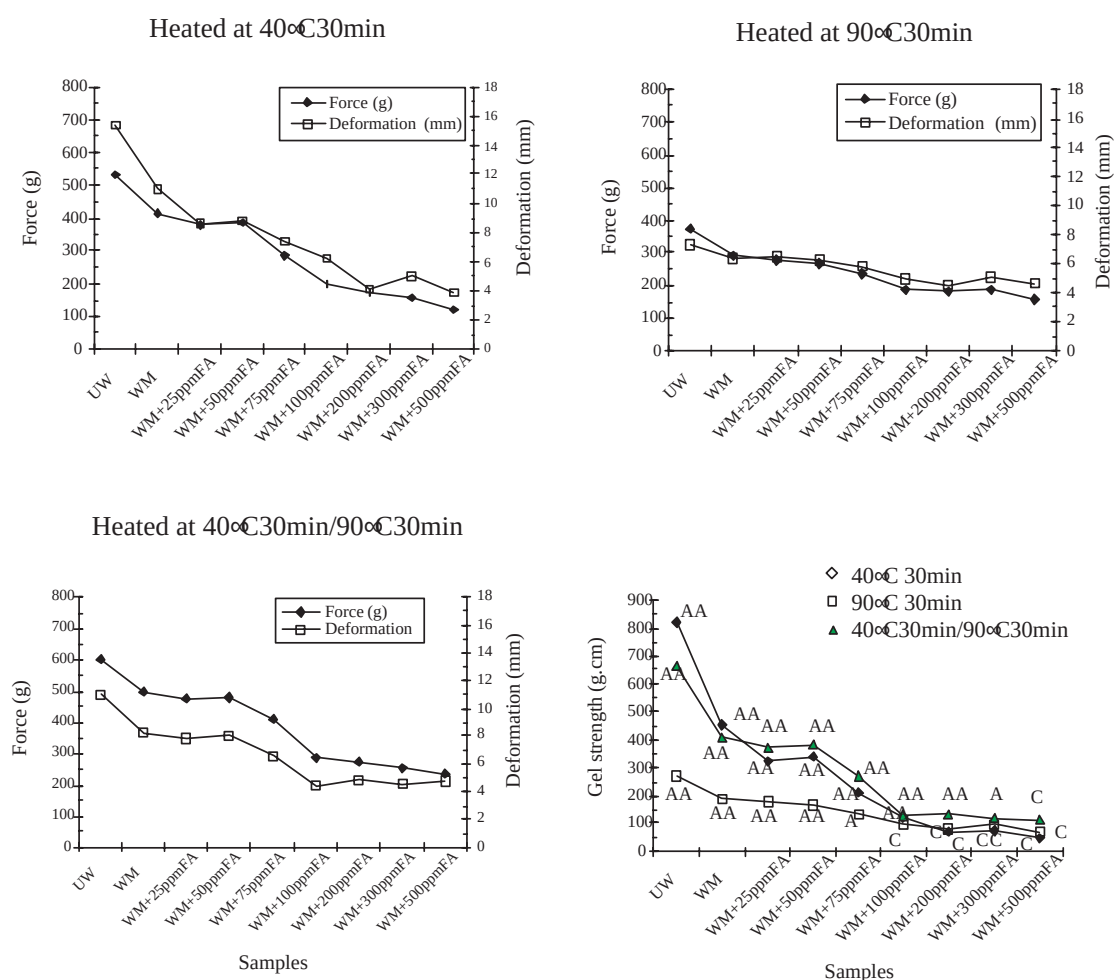


Figure 1 Force, deformation, gel strength and folding tests of dorab meat gel at various heating temperatures.

(UM:unwashed meat, WM:washed meat, FA:formalin solution)

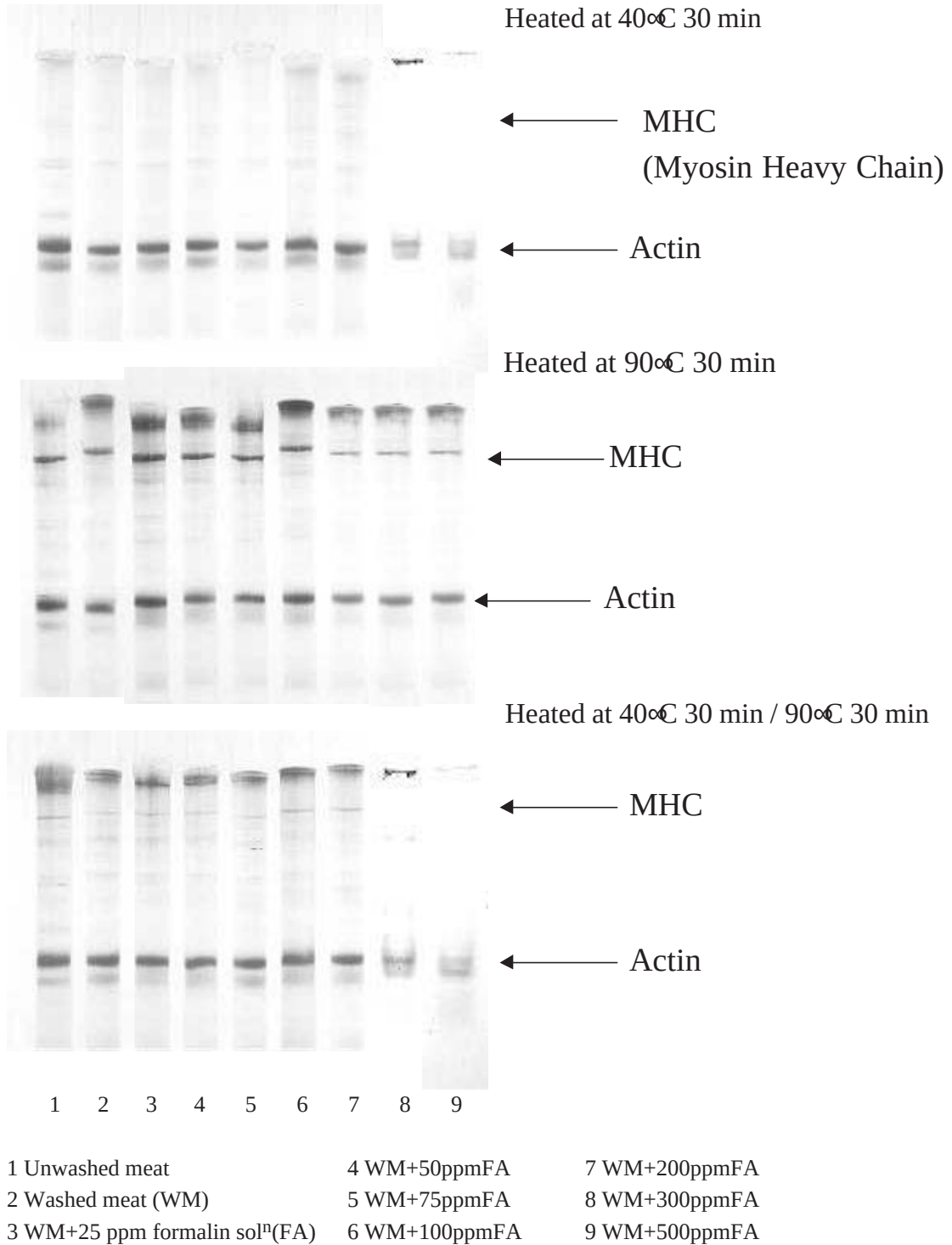


Figure 2 SDS-PAGE analysis of dorab meat gel at various heating temperatures.

denaturation of proteins (Ang and Hultin, 1989). Sophonphong and Rungjiratananan (1993) concluded that FA content provided a freshness index of fresh lizard fish and also had a good relationship with its gel forming ability.

Figure 2 shows the SDS-PAGE patterns of meat gels heated at (1) 40°C 30 min, (2) 90°C 30 min, and (3) 40°C 30 min followed by 90°C 30 min of unwashed meat, washed meat and washed meat with the addition of several concentrations of formalin solution. Protein patterns of unwashed meat gels were similar to those of washed meat. According to the report by Numakura *et al.* (1990) three main bands appeared on the SDS-PAGE pattern of surimi meat gels were assigned to cross-linked MHC (CMHC) band at the top of the disk gel, the second to MHC and the bottom to actin. The MHC bands were apparent in samples of meat gels heated at 90°C while those of meat gels heated at 40°C and 40°C followed by 90°C almost disappeared. Moreover, the CMHC bands were observed from both of meat gels heated at 90°C and 40°C followed by 90°C but were not observed in

that of 40°C. The disappearance of CMHC bands in SDS-PAGE pattern of salted ground meat heated at 40°C were found in Threadfin bream (Lee *et al.*, 1990) and Walleye pollack (Numakura *et al.*, 1990). This occurrence could be attributed to the degree of polymerization of CMHC which affected to its solubility and electrophoretic behavior (Nowsad *et al.*, 1993). Therefore, it is likely that CMHC in the samples of 40°C 30 min was not dissolved in the SDS-urea-mercaptoethanol buffer. After the addition of formalin solutions at various concentrations, MHC bands of dorab meat gels disappeared at all heating temperatures. This result correlated to the decrease of their gel forming ability. Especially in washed dorab meat gels addition with 300 and 500 ppm formalin solution, both MHC and Actin bands absolutely disappeared. It seems likely that the action of formaldehyde and heating affected the conformation of the myofibrillar proteins of both actin and MHC.

Table 2 Dimethylamine and formaldehyde concentrations of dorab meat.

Sample	DMA (ppm)	Total FA1/ (ppm)	Free FA (ppm)	Combined FA2/ (ppm)
Dorab meat (Unwashed meat, UW)	16.82	36.08	10.01	26.08
Washed dorab meat (WM + 0 ppm FA)	17.92	38.43	13.55	24.88
WM + 25 ppm FA	17.6	62.75	16.5	46.25
WM + 50 ppm FA	17.64	87.84	17.13	70.71
WM + 75 ppm FA	17.59	112.73	16.82	95.91
WM + 100 ppm FA	17.73	138.03	18.93	119.1
WM + 200 ppm FA	17.03	236.53	30.43	206.1
WM + 300 ppm FA	17.32	337.15	61.92	275.23
WM + 500 ppm FA	17.67	537.9	81.94	455.96

1/ Total FA = Estimated total FA after formalin addition

2/ Combined FA = Total FA - Free FA

CONCLUSION

Formaldehyde, free FA and combined FA at concentrations over 100 ppm, 20 ppm and 70 ppm, respectively, affected the gel forming ability of dorab meat.

The gel strength of dorab meat gel heated at 40°C and 40°C/90°C did not depend on disulfide linkages.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Hiroyasu Oka (Industrial Research Center of Ehime Prefecture, Matsuyama, Ehime, Japan) for his helpful suggestions. Thanks also go to the staff of Fishery Technological Development Institute for their technical assistance.

LITERATURE CITED

- Amano, K., K. Yamada, and M. Bito. 1963. Detection and identification of formaldehyde in gadoid fish. *Bull. Jap. Soc. Sci. Fish.* 29 (7) : 695-701.
- Ang, J.F. and H.O. Hultin. 1989. Denaturation of cod myosin during freezing after modification with formaldehyde. *J. Food Sci.* 54 : 814-818.
- AOAC. 1990. *Official Methods of Analysis*. 15th ed. Association of Official Analytical Chemists. Arlington, Virginia. pp. 69-81, 429-431, 449-451.
- Boeri, R.L., M.E. Almandos, A.S. Ciarlo, and D.H. Giannini. 1993. Formaldehyde instead of dimethylamine determination as a measure of total formaldehyde formed in frozen Argentine hake (*Merluccius hubbsi*). *Int. J. Food Sci. Tech.* 28 : 289-292.
- Dyer, W.J. and Y.A. Mounsey. 1945. Amines in fish muscle II. Development of trimethylamine and other amines. *J. Fish. Res. Bd. Can.* 6 (5) : 359-367.
- Hasegawa, H. 1992. Laboratory manual on analytical methods and procedures for fish and fish products. 2nd ed. Marine Fisheries Research Department, Southeast Asian Fisheries Development Centre, Singapore. pp. B-3.1 – B-3.7.
- Hiraoka, Y., T. Kan, K. Hirano, M. Kurono, A. Hashimoto, and H. Oka. 1995. Utilization of Tokage eso and Tachiuo for surimi industry, pp.110-120. *In Report of Ehime Industrial Technology*, No 31. (In Japanese)
- Lee, N., N. Seki, N. Kato, N. Nakagawa, S. Terui and K. Arai. 1990. Gel forming ability and cross-linking ability of myosin heavy chain in salted meat paste from threadfin bream. *Nippon Suisan Gakkaishi* 56 : 329-336.
- Marine Fisheries Research Department (MFRD). 1988. *Handbook on the Processing of Frozen Surimi and Fish Jelly Products in Southeast Asia*. Marine Fisheries Research Department, Southeast Asian Fisheries Development Centre, Singapore. 30 p.
- Nowsad, A. AKM., S. Kanoh, and E. Niwa. 1993. Electrophoretic behavior of cross-linked myosin heavy chain in suwari gel. *Nippon Suisan Gakkaishi* 59 : 667-671.
- Nozaki, Y., R. Kanazu, and Y. Tabata. 1978. Freezing storage of lizard fish for kamaboko preparation. *Refrigeration* 53 : 473-480.
- Numakura, T., I. Kimura, K. Toyoda, and T. Fujita. 1990. Temperature-dependent changes in gel strength and myosin heavy chain of salted-ground meat from walleye pollack during setting. *Nippon Suisan Gakkaishi* 56 : 2035-2043.
- Owusu-Ansah, Y.J. and H.O. Hultin. 1986. Chemical and physical changes in red hake fillets during frozen storage. *J. Food Sci.* 51 : 1402-1406.
- Sophonphong, K. and S. Rungjiratananan. 1993. Effects of pyrophosphate leaching on gel forming ability of lizard fish. Special Fellowship Report. Marine Fisheries Research Department, Southeast Asian Fisheries

- Development Center, Singapore. 31 p.
- Tunhun, D., S. Kanont, M. Chaiyawat, and N. Raksakulthai. 1996. Detection of illegal addition of formaldehyde to fresh fish. *ASEAN Food J.* 11 : 74-77.
- Weber, K. and M. Osborn. 1969. The reliability of molecular weight determination by dodecylsulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244 : 4406-4412.
- Yasui, A. and P.Y. Lim. 1987. Changes in chemical and physical properties of lizard fish meat during ice and frozen storage. *Nippon Shokuhin Kogyo Gakkaishi* 34 (1) : 54-60.
-
- Received date : 8/08/01
Accepted date : 28/09/01