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Contributed Paper

Physicochemical Instability of Muscles from Two Species of Scad During Iced Storage

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ABSTRACT

Physicochemical changes of oxeye scad (*Selar boops*) and shrimp scad (*Alepes djedaba*) whole muscles were investigated during ice storage (0°C) for 15 days. Muscle pHs of both species tended to increase throughout the storage period and shrimp scad muscle had a higher pH value at all time points ($p<0.05$). The decreases in Ca^{2+}-ATPase activity, protein solubility and sulfhydryl group content with a concomitant increase in disulfide bond formation were found in both species when the storage time increased ($p<0.05$). The proteolytic degradation pattern of both species, analyzed by SDS-PAGE, revealed that myosin heavy chain (MHC) was negligibly hydrolyzed but an increased trichloroacetic acid (TCA)-soluble peptide content was markedly found throughout the storage time ($p<0.05$). Breaking force of kamaboko gel of surimi prepared from both species decreased as storage time progressed ($p<0.05$) but a marked reduction in breaking force was noticeable in shrimp scad ($p<0.05$). Therefore, extended storage in ice seemed to have a negative effect on the stability of muscle protein as well as the gel strength of surimi processed from aged fish. In addition, the physicochemical changes of fish muscle during iced storage were species-dependent.

Keywords: oxeye scad, shrimp scad, muscle, iced storage, surimi

1. INTRODUCTION

Demand for fish is increasing due to the increase in population. Oxeye scad and shrimp scad are abundant dark-fleshed fish species commonly caught in Southern Thailand, especially in the Thasala coast of Nakhon Si Thammarat. During handling, fish usually deteriorate caused by microorganisms and chemical reactions resulting in the degradation and denaturation of myofibrillar proteins and the loss in their functional properties. Deterioration of fish during iced storage depends on many factors including fish species, storage temperature, time and enzymatic degradation [1]. The degree of denaturation varies, depending on fish species [2]. Denaturation and proteolytic degradation during iced storage lead to the loss in gel-forming ability of muscle protein [3]. This study aimed to investigate the physicochemical instability of muscles from oxeye scad and

shrimp scad and gel-forming ability of surimi from both species during iced storage.

2. MATERIALS AND METHODS

Oxeye scad and shrimp scad with an average weight of 110-120 g and 125-140 g, respectively, were obtained from the fishing port in Thasala, Nakhon Si Thammarat. Oxeye scad (50 kg) and shrimp scad (50 kg), off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the School of Agricultural Technology, Walailak University, Thasala Nakhon Si Thammarat within 1 h. Whole oxeye scad and shrimp scad were kept in ice with a fish/ice ratio of 1:2 (w/w). The fish were placed and distributed uniformly between the layers of ice. The box containing fish and ice was kept at 4°C for 15 days. To maintain the ice content, melted ice was removed and replaced with an equal amount of ice every 2 days. Average of inner temperature of fish measured by Testo 104 thermometer was 4.14±0.26 and4.22±0.24°C for oxeye scad and shrimp scad, respectively.

During storage, 1 kg of fish was randomly taken as the composite sample at days 0, 3, 6, 9, 12 and 15 for analyses. The fish samples were washed and filleted. The whole muscle was collected, minced to uniformity and used for analyses. The pH, protein solubility and TCA-soluble peptide was determined by the method of Benjakul and others [4], Benjakul and Bauer [5] and Morrissey and others [6], respectively. Ca^{2+}-ATPase activity, reactive sulfhydryl (SH) and disulfide bond content of natural actomyosin (NAM) was determined according to the method of Benjakul and others [4], Ellman [7] and Thannhauser and others [8], respectively. The protein patterns of whole muscle were analyzed by SDS-PAGE [9] under reducing condition. To prepare surimi by conventional washing process, whole fish mince was washed with cold water (4°C) [10] using a water/mince ratio of 3:1 (v/w). The mixture was stirred gently for 10 min in a cold room (4°C) and the washed mince was filtered with a layer of nylon screen. Washing was performed three times. Finally, the washed mince was centrifuged at 700 xg for 15 min using a basket centrifuge. The washed mince was added with 4% sucrose and 4% sorbitol mixed well and frozen using an air-blast freezer. The frozen samples referred to as 'surimi' were kept at -18°C until used. To prepare the gels, the frozen surimi samples were thawed at 4°C for 3-4 h until the core temperature reached 0°C. The samples were then cut into small pieces and the moisture content was adjusted to 80% by the addition of iced water. The samples were added with 2.5% (w/w) NaCl and chopped for 5 min in a walk-in cold room at 4°C to obtain the homogeneous sol. The sol was stuffed into a polyvinylidine casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly. The sol was then incubated at 40°C for 30 min, followed by heating at 90°C for 20 min and cooled in iced water for 30 min. This sample was referred to as 'kamaboko gel' [10]. The gels were stored for 24 h at 4°C prior to analysis. Breaking force and deformation of surimi gel prepared from both species during storage were measured using a TA-XT2i texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) with a spherical plunger (diameter 5 mm, depression speed of 60 mm/min). For the stastistical analysis, data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test. The statistical analysis was performed using Statistical Package for Social Science (SPSS 10.0 for windows).

3. RESULTS AND DISCUSSION

3.1 pH

The changes in muscle pH of scads during storage in ice are shown in Figure 1. The initial pH values of oxeye scad and shrimp scad stored in ice were 6.38±0.04 and 6.66±0.02, respectively. Variations amongst the initial values of pH may due to the species, diet, level of activity and other factors [11]. During the storage period the pH of oxeye scad muscle was consistently lower than that of shrimp scad muscle. The differences in pH changes between the two species might be due to the differences in the glycolysis rate [12], buffering capacity and microbial count of those muscles. The activity of enzymes converting glycogen into lactic acid might be different between two species. Lactic acid, generated in anoxic conditions from glycogen, is the principal factor in lowering the post mortem pH in the fish muscles [10]. The increase in pH during extended storage was mainly due to the decomposition of nitrogenous compounds caused primarily by microbial activity [13].

During the first 6 days of storage, the pH values of both species increased markedly. At day 9, decrease in the pH values of two species was observed. The rapid decline in pH could be the result of the accumulation of lactic acid [14]. However, the pH values of both fish species increased significantly from day 9 to day 15 ($p<0.05$). This was probably due to the accumulation of basic compounds such as ammonia and TMA, mainly derived from microbial action [15].

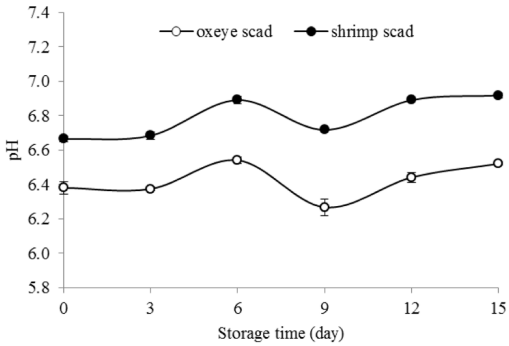


Figure 1. Changes in pH of oxeye scad and shrimp scad muscles during iced storage.

3.2 Ca^{2+}-ATPase Activity

The Ca^{2+}-ATPase activities of the NAM extracted from oxeye scad muscle decreased with increasing storage time ($p<0.05$) (Figure 2). The decreasing rate of Ca^{2+}-ATPase activity was much greater in oxeye scad muscle than in shrimp scad muscle. For shrimp scad, the Ca^{2+}-ATPase activity remained constant for the first 12 days and decreased sharply on day 15. The results indicated that myosin underwent some conformational changes during iced storage. Ca^{2+}-ATPase has been used as an indicator of myosin integrity [4]. The loss in Ca^{2+}-ATPase was possibly associated with the proteolysis and denaturation of myosin molecule [16]. Differences in protein integrity among fish species were possibly caused by different susceptibility to denaturation of muscle protein during handling. As the storage time increased, the marked decrease in Ca^{2+}-ATPase activity was observed. The decreasing rate of Ca^{2+}-ATPase activity varied, depending on species [17].

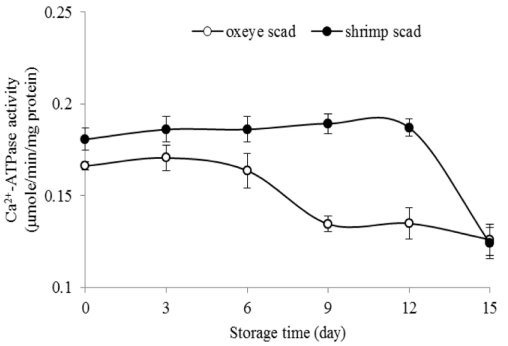


Figure 2. Ca^{2+}-ATPase activity of NAM extracted from oxeye scad and shrimp scad muscles during iced storage.

3.3 SH and Disulfide Bond Contents

A sharp decrease in SH content was observed in the first 3 days of storage, particularly in shrimp scad muscle. Greater decrease in SH content was observed in shrimp scad than oxeye scad ($p<0.05$) (Figure 3(a)). Conversely, SH contents of oxeye scad increased gradually within the first 3 days. Thereafter, SH content decreased ($p<0.05$) as the storage time increased (Figure 3(a)). From this result, the decrease in SH content was in agreement with the increase in disulfide bond content (Figure 3(b)). The accelerated denaturation of myosin molecules, especially the conformational changes, in which the reactive SH groups were exposed to oxidation, might result in increased disulfide bond formation. However, the changes in disulfide bond varied, depending on species [3].

3.4 Protein Solubility

Protein solubility of oxeye scad and shrimp scad muscles decreased gradually during iced storage ($p<0.05$) (Figure 4). It has been reported that a progressive decrease in solubility of myofibrillar proteins during storage was due to protein aggregation [18]. Oxeye scad showed a greater decrease in protein solubility than shrimp scad, particularly after day 9. The decrease in solubility was most likely associated with the formation of disulfide bonds (Figure 3b). Disulfide bonds are associated with the crosslinking of myosin heavy chain contributing to the formation of high molecular weight polymers and aggregates [19]. A decrease in protein solubility during prolong storage of fish muscle could be related to the possible unfolding of myofibrillar proteins exposing the hydrophobic groups to the exterior [20-21]. Another possible contributing factor could be the formation of the cross bridge between myosin and actin in the post-rigor muscle [22]. Presence of structural proteins like M-line and Z-line proteins, which are responsible for the integrity of muscle structure, can cause a decrease the solubilization of myofibrillar proteins in fresh fish [22-23]. The solubility characteristics related to the protein surface composition, which is responsible for the protein-solvent interaction and hence solubilization [24].

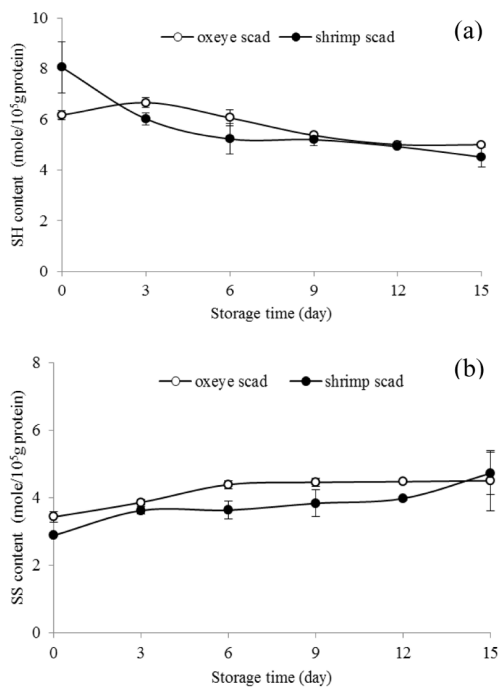


Figure 3. Changes in reactive SH (a) and disulfide bond (b) contents of NAM extracted from oxeye scad and shrimp scad during iced storage.

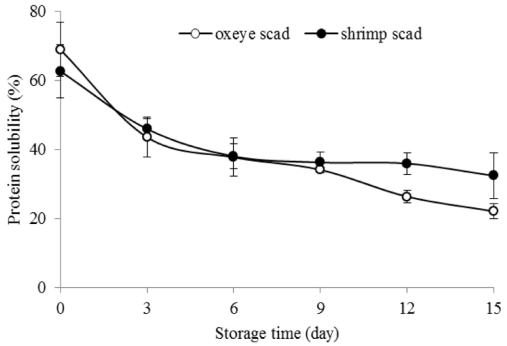


Figure 4. Changes in protein solubility of oxeye scad and shrimp scad muscles during iced storage.

3.5 TCA-soluble Peptides and Protein Pattern

TCA-soluble peptides of both species increased throughout 15 days of iced storage ($p<0.05$), suggesting the autolytic degradation of fish proteins (Figure 5). Oxeye scad muscle had more TCA-soluble peptides than shrimp scad muscle. TCA-soluble peptide observed in both samples were conversely proportional, with MHC and actin remaining in the samples (Figure 6). It can be noted that not only MHC and actin were degraded during storage but another muscle proteins also underwent degradation. Therefore, the increase in TCA-soluble peptide without marked changes in MHC and actin visualized by SDS-PAGE was noticeable. In addition, the amount or the size of the protein fragments might be too small to be detected owing to the sensitivity and resolution limits of the present electrophoretic analysis [25].

The initial TCA soluble peptides might indicate the endogenous oligopeptides in both samples as well as the degradation products generated during post-harvest handling [4]. The increase in the contents of TCA-soluble peptides during storage might indicate the activity of endogenous and microbial proteases [25]. In general, a slight increase in TCA-soluble peptide contents was found for shrimp scad, while a higher rate of increase was found for oxeye scad ($p<0.05$), suggesting the continuous occurrence of proteolysis during storage. This finding is somewhat similar to those found in lizardfish [3] sea bass [26] and cod muscles [27] in which no marked changes in the electrophoretic profiles were observed whereas the TCA-soluble peptide contents increased significantly.

Autolytic proteolysis through the action of endogenous enzymes may contribute to the deterioration of fish flesh [28] but this phenomenon is preferred in some fish fermentation [29]. The differences in fish species and the temperatures of their normal habitat may contribute to the differences in postmortem degradation of various muscle proteins [28].

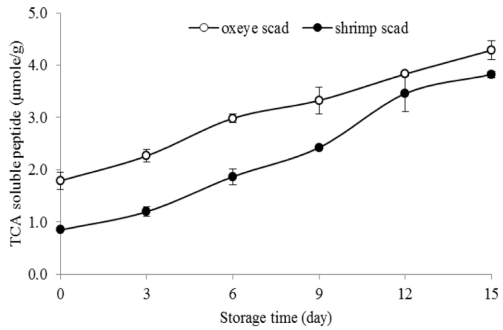


Figure 5. TCA-soluble peptides of oxeye scad and shrimp scad muscles during iced storage.

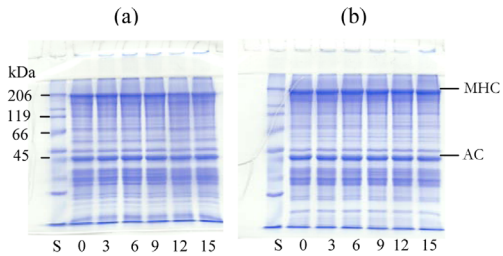
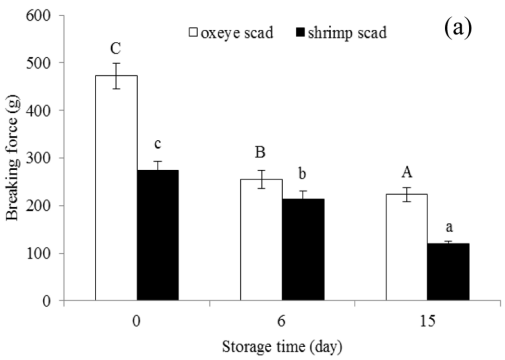


Figure 6. SDS–PAGE pattern of muscle proteins from oxeye scad (a) and shrimp scad (b) during iced storage under reducing condition. Numbers designate storage time (days). S = standard marker, MHC = myosin heavy chain and AC = actin.

3.6 Textural Properties of Surimi Gels

Breaking force and deformation of surimi gels prepared from oxeye scad and shrimp scad stored in ice are shown in Figure 7. When the storage time increased breaking force of surimi gel from both species decreased markedly ($p<0.05$). Greater decrease in breaking force was found in surimi gel prepared from shrimp scad ($p<0.05$). Continuous decreases in deformation was observed when storage time increased ($p<0.05$). However, after day 6, no marked difference in deformation was found in surimi gel from oxeye scad.

This result was coincidental with the increase in protein denaturation and degradation with increasing storage time [3]. During the denaturation or degradation processes, the hydrophobic and hydrogen bonds buried in the interior of the protein molecule become exposed and broken from their native arrangement with consequent conformational changes in coiled or helical sections of the peptide chain [30]. Denatured proteins could form the aggregates where the imbalance between protein-protein and protein-water interactions took place. Therefore, the gel strength and deformation of stored fish were inferior to those prepared from fresh fish.



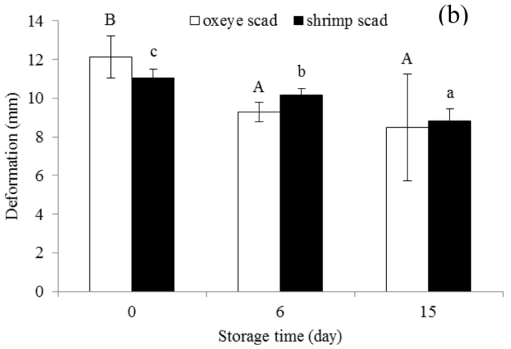


Figure 7. Breaking force (a) and deformation (b) of surimi gels from oxeye scad and shrimp scad during 15 days of iced storage.

3.7 Whiteness

Whiteness of surimi gels from both species generally decreased as the storage time increased ($p<0.05$; Figure 8). Normally, gels from shrimp scad had higher whiteness than had oxeye scad at all time points. Decrease in whiteness was probably due to the oxidation of residual heme pigments in fish muscle, particularly myoglobin and hemoglobin [3]. Additionally, lipid oxidation products such as aldehyde formed during storage might be interacted with muscle proteins via the Maillard reaction to form brown pigment [31]. As a consequence, surimi gel produced from stored fish showed a lower whiteness compared with that did from fresh fish [3].

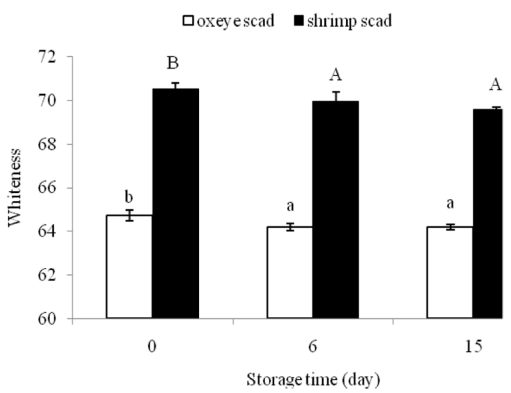


Figure 8. Whiteness of surimi gels from oxeye scad and shrimp scad during 15 days of iced storage.

4. CONCLUSION

Extended storage in ice seemed to have a negative effect on the stability of scad muscle proteins as well as the gel strength of surimi processed from stored fish. In addition, the physicochemical changes of fish muscle during iced storage were species-dependent.

ACKNOWLEDGEMENT

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REFERENCES

[1] Badii F. and Howell N. K., A comparison of biochemical changes in cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) fillets during frozen storage, *J. Sci. Food Agric.*, 2001; **82**: 87-97.

[2] Seo H., Endo Y., Fujimoto K., Moku M. and Kawaguchi K., Denaturation of myofibrillar protein in myoctophid fish during refrigeration and freezing storage, *Fish. Sci.*, 1997; **63**: 839-840.

[3] Benjakul S., Visessanguan W. and Tueksuban J., Changes in physico-chemical properties and gel-forming ability of lizardfish (*Saurida tumbil*) during post-mortem storage in ice, *Food Chem.*, 2003; **80**: 535-544.

[4] Benjakul S., Seymour T.S., Morrissey M.T. and An H., Physicochemical changes in Pacific whiting muscle proteins during iced storage, *J. Food Sci.*, 1997; **62**: 729-733.

[5] Benjakul S. and Bauer F., Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze-thaw cycles, *J. Sci. Food Agric.*, 2000; **80**: 1143-1150.

[6] Morrissey M. T., Wu J. W., Lin D. D. and An H., Protease inhibitor effects on torsion measurements and autolysis of Pacific whiting surimi, *J. Food Sci.*, 1993; **58**: 1050-1054.

[7] Ellman G. L., Tissue sulfhydryl groups, *Arch. Biochem. Biophys.,* 1959; **82**: 70-77.

[8] Thannhauser T. W., Konishi Y. and Scheraga H. A., Analysis for disulfide bonds in peptides and proteins, *Meth. Enzymol.*, 1987; **143**: 155-161.

[9] Laemmli U. K., Cleavage of structural protein during the assembly of the head bacteriophage T4, *Nature*, 1970; **227**: 680-685.

[10] Chaijan M., Benjakul S., Visessanguan W. and Faustman C., Characteristics and gel properties of muscles from sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) caught in Thailand, *Food Res. Int.*, 2004; **37**: 1021-1030.

[11] Li X., Li J., Zhu J., Wang Y., Fu L. and Xuan W., Postmortem changes in yellow grouper (*Epinephelus awoara*) fillets stored under vacuum packaging at 0°C, *Food Chem.*, 2011; **126**: 896-901.

[12] Love R. M., *The Food Fish: Their Intrinsic Variation and Practical Implications*, UK: Farrand Press, London, 1988.

[13] Hernández M. D., López M. B., Álvarez A., Ferrandini E., García B. and Garrido M. D., Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage, *Food Chem.*, 2009; **114**: 237-245.

[14] Pacheco-Aguilar R., Marquez-Ríos E., Lugo-Sánchez M. E., García-Sanchez G., Maeda-Martínez A. N. and Ocaño-Higuera V. M., Postmortem changes in the adductor muscle of Pacific lions-paw scallop (*Nodipecten subnodosus*) during ice storage, *Food Chem.*, 2008; **106**: 253-259.

[15] Benjakul S., Visessanguan W., Riebroy S., Ishizaki S. and Tanaka M., Gel-forming properties of surimi produced from bigeye snapper, *Priacanthus tayenus* and *P. macracanthus*, stored in ice, *J. Sci. Food Agric.*, 2002; **82**: 1442-1451.

[16] Ouali A. and Valin C., Effect of muscle lysosomal enzymes and calcium activated neutral proteinase on myofibriallar ATPase activity: relationship with aging changes, *Meat Sci.*, 1981; **5**: 233-245.

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

[18] Matsumoto J. J. and Noguchi S. F., Crystallization of Protein in Surimi; in Lanier T. C., and Lee C. M., eds., *Surimi Technology*, Marcel Dekker, New York, 1992: 357-388.

[19] Benjakul S. and Sutthipan N., Muscle changes in hard and soft shell crabs during frozen storage, *LWT - Food Sci.Tech.,* 2009; **42**:723-729.

[20] Sarma J., Srikar L. N. and Reddy G. V., Effect of ice storage on the functional properties of pink perch and oil sardine meat, *J. Sci. Food Agric.*, 1999; **79(2):** 169-172.

[21] Srikar L. N., Protein denaturation in iced storage and frozen marine fish: changes in muscle proteins of croaker fish during ice and frozen storage, *Mysore J. Agric. Sci.,* 1979; **13**: 78-82.

[22] Offer G. and Trinick J., On the mechanism of water holding in meat: the swelling and shrinking of myofibrils, *Meat Sci.*, 1983; **8**: 245.

[23] Xiong Y. L. and Brekke C. J., Changes in protein solubility and gelation properties of chicken myofibrils during storage, *J. Food Sci.*, 1989; **54**: 1141.

[24] Bigelow C. C., On the average hydrophobicity of proteins and the relation between protein structure, *J. Theor. Biol.*, 1967; **16**: 187.

[25] Liu D., Liang L., Xia W., Regenstein J. M. and Zhou P., Biochemical and physical changes of grass carp (*Ctenopharyngodon idella*) fillets stored at -3 and 0°C, *Food Chem.*, (in Press).

[26] Verrez-Bagnis V., Ladrat C., Morzel M., Noel J. and Fleurence J., Protein changes in post mortem sea bass (*Dicentrarchus labrax*) muscle monitored by one- and two-dimensional gel electrophoresis, *Electrophoresis*, 2001; **22**: 1539-1544.

[27] Kjærsgård I. and Jessen F., Proteome analysis post mortem changes in cod (*Gadus morhua*) muscle proteins, *J. Agric. Food Chem.*, 2003; **51**: 3985-3991.

[28] Delbarre-Ladrat C., Chéret R., Taylor R. and Verrez-Bagnis V., Trends in postmortem aging in fish: Understanding of proteolysis and disorganization of the myofibrillar structure, *Crit. Rev. Food Sci. Nutr.,* 2006; **46**: 409-421.

[29] Udomthawee K., Chunkao K., Phanurat A. and Nakhonchom K., Protein, calcium and phosphorus composition of fermented fish in the lower Mekong basin, *Chiang Mai J. Sci.*, 2012; **39(2)**: 327-335.

[30] Morawezt H., Conformational transitions in macromolecules, *Adv. Protein Chem.*, 1972; **26**: 243-248.

[31] Saeed S., Fawthrop S. A. and Howell N. K., Electron spin resonance (ESR) study on free-radical transfer in fish lipid-protein interactions, *J. Sci. Food Agric.*, 1999; **79**: 1809-1816.