PHYSICOCHEMICAL INSTABILITY OF MUSCLES FROM TWO SPECIES OF SCAD DURING ICED STORAGE

Chantira Wongwichian [a], Manat Chaijan *[a] and Sappasith Klomklao [b]

[a] Division of Agro-Industry, School of Agricultural Technology, Walailak University, Thasala, Nakhon Si Thammarat, 80161 Thailand

[b] Department of Food Science and Technology, Faculty of Technology and Community Development, Thaksin University, Phattalung Campus, Phattalung 93110, Thailand

* Author for correspondence; e-mail: cmanat@wu.ac.th

Abstract

Physicochemical changes of oxeye scad (*Selar boops*) and shrimp scad (*Alepes djedaba*) whole muscles during iced storage for 15 days were investigated. 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 Ca²⁺-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 hydrolyzed continuously throughout the storage time corresponding to an increased trichloroacetic acid (TCA)-soluble peptide content (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.

1. INTRODUCTION

Fish have become increasingly demanded 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 varied, depending on fish species [2]. Denaturation and proteolytic degradation during iced storage led to the loss in gel-forming ability of muscle protein [3]. Thus, this study aimed to investigate the physicochemical instability of muscles from oxeye scad and shrimp scad during iced storage.

2. MATERIALS AND METHODS

Oxeye scad with an average weight of 110-120 g and shrimp scad with an average weight of 125-140 g obtained from the fishing port in Thasala along the coast of the Gulf of Thailand were used for this study. 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. 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, chopped 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. Ca2+-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], Thannhauser and others [8], respectively. The protein patterns of whole muscle were analyzed by SDS-PAGE [9] under reducing and non-reducing conditions. Breaking force and deformation of surimi gel prepared from both species during storage were measured using the texture analyzer equipped with a spherical plunger (diameter 5 mm, depression speed of 60 mm/min).

3. RESULTS AND DISCUSSION

3.1 pH

The changes in muscle pH of scads during storage in ice are shown in Fig. 1. During the initial

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 chemical reaction rate, 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]. However, the pH values of both fish species increased significantly (p<0.05) during ice storage. This is probably due to the accumulation of basic compounds such as ammonia compound and TMA, mainly derived from microbial action [11].

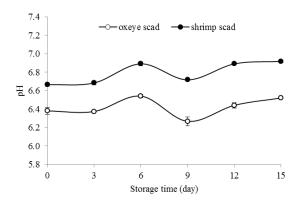


Figure 1. Changes in pH of oxeye scad and shrimp scad muscles during iced storage. Bars indicate SD from triplicate determinations.

3.2 Ca²⁺-ATPase activity

The Ca2+-ATPase activities of the NAM extracted from oxeve scad and shrimp scad muscles decreased with increasing storage time (p < 0.05) (Fig. 2). The decreasing rate of Ca²⁺-ATPase activity was much greater in oxeye scad muscle than in shrimp scad muscle. For shrimp scad, the Ca2+-ATPase activity remained constant for the first 12 days and decreased sharply on day 15. The results indicated that myosin underwent some conformation changes during iced storage. Ca2+-ATPase has been used as an indicator of myosin integrity [4]. The loss in Ca²⁺-ATPase was possibly associated with the proteolysis of myosin molecule [12]. 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 Ca2+-ATPase activity was observed. The decreasing rate of Ca²⁺-ATPase activity varied, depending on species [13].

3.3 SH and disulfide bond contents

SH contents of NAM from both species decreased gradually during iced storage (p<0.05)

(Fig. 3(a)). 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. From this result, the decrease in SH content was in agreement with the increase in disulfide bond content (Fig. 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].

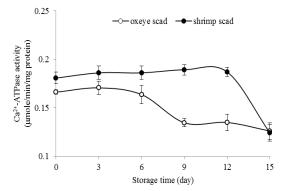


Figure 2. Ca²⁺-ATPase activity of NAM extracted from oxeye scad and shrimp scad muscles during iced storage.

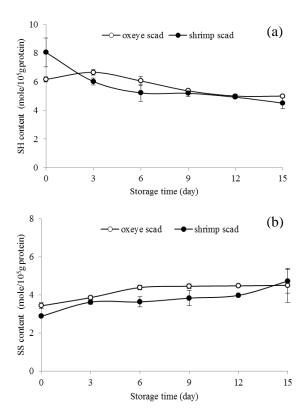


Figure 3. Changes in reactive SH (a) and disulfide bond (b) contents of NAM extracted from oxeye scad and shrimp scad during iced storage.

3.4 Protein solubility

Protein solubility (in 0.6 M KCl) of oxeve scad and shrimp scad muscles decreased gradually during iced storage (p<0.05) (Fig. 4). 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 (Fig. 3). The decrease in solubility of protein has been used as a marker of oxidative deterioration of muscle protein [14]. Thermodynamically, a decrease in protein solubility is the result of a shift from a balance of protein intermolecular interaction and protein-water interaction, resulting in a situation where protein intermolecular interaction is strengthened, while protein water interaction is weakened [15].

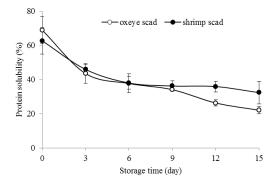


Figure 4. Changes in protein solubility of oxeye scad and shrimp scad muscles during iced storage.

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 (Fig. 5). Oxeye scad muscle had more TCA-soluble peptides than shrimp scad muscle. TCA-soluble peptide observed in both samples were conversely proportional, with myosin heavy chain remaining in the sample (Fig. 6). SDS-PAGE patterns revealed that myosin heavy chain was much more susceptible to hydrolysis than was actin. This result was in agreement with Benjakul and others [3] who reported that myosin heavy chain was more prone to proteolytic degradation than other muscle proteins, such as actin, troponin and tropomyosin.

3.6 Textural properties of surimi gels

Fish kept in ice for different times were washed, filleted manually and minced to uniformity. The surimi was prepared by washing fish mince with cold water (4 °C) using a water/mince ratio of 3:1 (v/w) for three times. The moisture of the washed mince was adjusted to 80%. For gel preparation, surimi was ground in the presence of 2.5% (w/w) NaCl to obtain the homogenous sol. The sol was incubated at

40 °C for 30 min and subsequently heated at 90 °C for 20 min. The resulting gel was referred to as 'kamaboko gel' [11].

Breaking force and deformation of surimi gels prepared from oxeye scad and shrimp scad stored in ice are shown in Fig. 7. When the storage time increased breaking force of surimi gel from both species decreased markedly (p<0.05). The rate of decrease in breaking force was higher in shrimp scad than oxeye scad (p<0.05). Continuous decreases in deformation was observed when storage time increased (p<0.05). The result was in agreement with the increase in protein denaturation and degradation with increasing storage time [3, 11]. However, after day 6, no marked difference in deformation was found in surimi gel from oxeye scad.

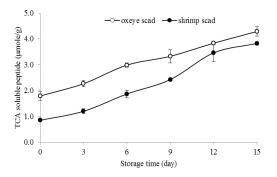
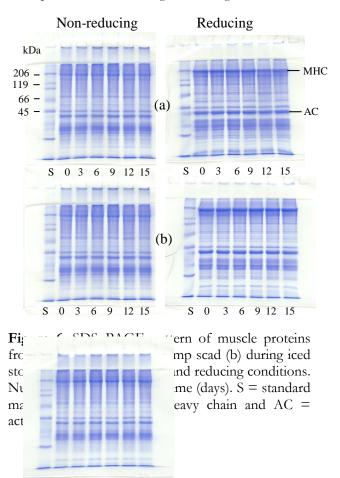


Figure 5. TCA-soluble peptides of oxeye scad and shrimp scad muscles during iced storage.



The decrease in breaking force and deformation was most likely associated with myosin heavy chain remaining in the sample (Fig. 6). The degradation of myosin resulted in an inferior gel network formation, causing a lower elasticity with poor water-holding capacity in the gel matrix [3].

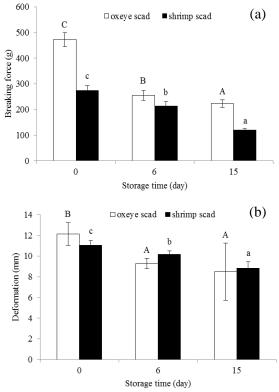


Figure 7. Breaking force and deformation of surimigels from oxeye scad and shrimp scad during 15 days of iced storage.

4. CONCLUSION

Muscle proteins of oxeye scad and shrimp scad underwent physicochemical changes during iced storage, leading to the loss in gel-forming ability. The degree of changes was governed by species and storage time.

ACKNOWLEDGEMENT

This work was supported by Walailak University Fund.

REFERENCES

- 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 Agr., 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 gelforming 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 Agr.*, 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. Archives of Biochemistry and Biophysics, *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] Sikorski Z. E., Kolakowska A. and Burt J. R., Postharvest biochemical and microbial changes; in Z. E. Sikorski, ed., *Seafood: Resources, nutritional* composition, and preservation, CRC Press, USA, 1990: 55–85.
- [11] 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 Agr.*, 2002; 82: 1442–1451.
- [12] 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.
- [13] 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.
- [14] Xiong Y. L. and Decker E. A., Alteration of muscle protein functionality by oxidative and antioxidative process, *J Muscle Food.*, 1995; 6: 139–160.
- [15] Vojdani F, Solubility; in G. M. Hall, ed., Method of testing protein functionality, Chapman & Hall, London, 1996: 11–60.