ELSEVIER

Food Chemistry 80 (2003) 535–544

Food Chemistry

www.elsevier.com/locate/foodchem

Changes in physico-chemical properties and gel-forming ability of lizardfish (*Saurida tumbil*) during post-mortem storage in ice

Soottawat Benjakul[a,*], Wonnop Visessanguan[b], Jiravadee Tueksuban[a]

[a]*Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand*
[b]*National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Klong 1, Paholyothin Road, Klong Luang, Pathumthani 12120, Thailand*

Received 7 August 2001; accepted 5 July 2002

Abstract

Changes in physico-chemical properties and gel-forming ability of lizardfish muscle (*Saurida tumbil*), stored in ice, were investigated up to 15 days. Heading and eviscerating, prior to iced storage, retarded myosin heavy chain degradation and formaldehyde formation. Additionally, denaturation of myosin and troponin was slightly impeded as monitored by the lower decrease in Ca^{2+}-ATPase and lower increase in Mg^{2+}–EGTA-ATPase, respectively. Gel-forming ability of surimi, prepared under different setting and/or heating conditions, decreased as storage time increased ($P<0.05$). However, superior breaking force and deformation of surimi gel, from headed/eviscerated fish, to that from whole fish was observed throughout the storage. Whiteness of surimi gel from headed/eviscerated fish was much higher than that from whole fish, especially when the storage time increased. Therefore, storage time and pretreatment were found to be crucial factors, determining the changes in physico-chemical properties and gel-forming ability of lizardfish during iced storage.

Keywords: Lizardfish; Muscle; Gelation; Iced storage; Degradation; Denaturation

1. Introduction

Functional properties of muscle protein are closely associated with the integrity of proteins. Denaturation and degradation, mainly, contribute to the loss of those functionalities. Gel-forming ability is a very important indication of functional and textural properties of fish muscle. However, there are several factors influencing the gelling properties of muscle protein, including species (Shimizu, Machida, & Takenami, 1981; Suwansakornkul, Itoh, Hara, & Obatake, 1993), death condition (Shimizu & Kaguri, 1986), maturation and spawning (Shimizu & Wendakoon, 1990) and freshness (Yean, 1993). Freshness is generally considered as the most crucial factor determining the final gel quality. Surimi gel quality is affected by time and temperature of the fish between capture and processing (Yean, 1993). Lower quality can be found in the gel made from the fish stored over time in ice. However, the rate of loss in gel strength appears to vary among species. Gel strength of kamaboko, made from lizard fish kept in ice for 3 days, was 50% of that made from fresh fish (Kurokawa, 1979). Acceptable quality surimi could be made from Northern squawfish stored up to 9 days (Lin & Morrissey, 1995), while good quality surimi could be produced from hoki stored in ice up to 10 days (MacDonald, Lelievre, & Wilson, 1990). Surimi gel quality can be influenced by many factors affecting protein structure. Severe proteolysis of myofibrillar proteins, caused by the endogenous proteinases in the muscle, is directly associated with poor gel quality (An, Peters, & Seymour, 1996). However, leakage of digestive enzymes also contributes to subsequent hydrolysis of muscle proteins. Therefore, pretreatment of fish, including heading and eviscerating prior to handling, can be another means to retard the deterioration caused by proteolysis (Benjakul, Visessanguan, Riebroy, Ishizaki, & Tanaka, in press).

Lizardfish (*Saurida* spp.) have been considered as a potential raw material for high-grade surimi in Japan,

* Corresponding author. Tel.: +66-74-226334; fax: +66-74-212889.
E-mail address: bsoottaw@ratree.psu.ac.th (S. Benjakul).

0308-8146/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0308-8146(02)00339-4

due to their high yield, white colour, good flavour and high gel-forming ability (Morrissey & Tan, 2000). Lizardfish are considered as a low-market-value fish in Southeast Asia due to their appearance and susceptibility to spoilage. They are not commonly consumed. As a consequence, large quantities are landed as trawl by-catch. Improper handling of fish, after catching, accelerates further reduction in its quality (Morrissey & Tan, 2000). In Thailand, lizardfish have been used to produce the low-grade surimi, which is mainly served for low-priced fishcake products. Lizardfish caught in Japan (*S. elongata* and *S. undosquamis*) exhibit excellent gel-forming ability, especially when fresh fish are used (Kurokawa, 1979; Suwansakornkul et al., 1993). However, gel strength of kamaboko made from lizardfish decreases rapidly during iced storage (Kurokawa, 1979). Yasui and Lim (1987) suggested that the decrease in gelling property of lizardfish during iced storage was due to the formation of formaldehyde and dimethylamine. The denaturation and degradation of myofibrillar proteins have been postulated to cause the loss of gel-forming ability of surimi produced from fish stored in ice for a longer period (Benjakul et al., in review). To alleviate the loss of gel quality associated with both chemical and microbiological deterioration, pretreatment of fish, such as deheading and eviscerating, might be a promising practice. Although lizardfish (*S. tumbil*) have been extensively used as raw material for surimi production in Thailand, little information regarding the changes in physico-chemical properties, or their relation to gelling ability, has been reported. The objectives of this study were to investigate the post-harvest changes in physico-chemical properties as well as gel-forming ability of lizardfish caught from the Gulf of Thailand and to study the effects of deheading/eviscerating on the retardation of those changes.

2. Materials and methods

2.1. Chemicals

Adenosine 5′-triphosphate (disodium salt), β-mercaptoethanol, acetylacetone, formaldehyde, ammonium acetate, 2,4,6-trinitrobenzenesulfonic acid (TNBS), sodium sulfite and L-leucine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid was purchased from Riedel-deHaen (Seelze, Germany). All chemicals for electrophoresis were obtained from Bio-Rad (Richmond, CA, USA).

2.2. Fish samples

Lizardfish (*Saurida tumbil*), caught off the Songkhla Coast along the Gulf of Thailand, were stored in ice and off-loaded within 8–12 h capture. Fish kept in ice were transported to the Department of Food Technology, Prince of Songkla University and washed with tap water. Fish were separated into two groups: whole fish and headed/eviscerated fish. After pre-treatment, the fish were washed thoroughly with tap water to remove the blood and viscera. Fish samples were kept in the styrene foam box containing crushed ice with a fish to ice ratio of 1:2 (w/w). The fish were placed between the layers of ice. The box was kept at room temperature (28–30 °C). To maintain the ice content, melted ice was removed and replaced with an equal amount of ice every 2 days. Fish were randomly taken every 3 days for analysis.

2.3. Determination of total volatile bases (TVB) and trimethylamine (TMA)

TVB and TMA contents were determined using the Conway microdiffusion assay according to the method of Conway and Byrne (1936). Fish meat (2 g) was extracted with 8 ml of 4% trichloroacetic acid (TCA). The mixtures were filtered using Whatman no. 41 and the filtrate was used for analysis. To determine the TMA content, formaldehyde was added to the filtrate to fix ammonia present in the sample. TVB and TMA were released after addition of saturated K_2CO_3 and diffused into the boric acid solution. The titration of solution was performed and the amount of TVB or TMA was calculated.

2.4. Determination of formaldehyde

Formaldehyde content in lizardfish sample was determined using Nash's reagent, as described by Amano, Yamada, and Bito (1963). Muscle (5 g) was mixed with 20 ml of 5% TCA and homogenised with a homogeniser (IKA Labortechnik, Malaysia). The homogenate was filtered with Whatman no. 41 filter paper. The residue was treated with 10 ml of 5% TCA, homogenised and filtered. The filtrate was combined and adjusted to pH of 6.0–6.5 using 0.1 N KOH or 1 N KOH. The resulting filtrate was made up to a final volume of 50 ml with distilled water. An aliquot (3 ml) of filtrate was mixed with 3 ml of acetylacetone reagent, thoroughly, and the mixture was kept at 60 °C for 15 min and cooled with running water. The absorbance was measured at 412 nm and the formaldehyde content was calculated from a standard curve.

2.5. Determination of Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity of actomyosin was determined, as described by Benjakul, Seymour, Morrissey, and An (1997). An aliquot (1 ml) of actomyosin solution (2.5–4 mg/ml) in 0.6 M KCl, pH 7.0, was mixed with 0.6 ml of 0.5 M Tris–maleate, pH 7.0, and 1 ml of 0.1 M $CaCl_2$.

Deionised water was added to make up a total volume of 9.5 ml. To the solution prepared, 0.5 ml of 20 mM ATP was added to initiate the reaction. The reaction mixture was incubated for 8 min at 25 °C and terminated by adding 5 ml of chilled 15% (w/v) TCA. The reaction mixture was then centrifuged at 3500×*g* for 5 min. The inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). The specific activity was expressed as μmoles inorganic phosphate (Pi) released/mg protein/min. A blank solution was prepared by adding chilled TCA prior to addition of ATP.

2.6. Determination of TCA-soluble peptides

TCA-soluble peptides were determined according to the method described by Morrissey, Wu, Lin, and An (1993). Fish muscle (3 g) was homogenised with 27 ml of 5% TCA (w/v). The homogenate was kept in ice for 1 h and centrifuged at 5000×*g* for 5 min. Soluble peptides in the supernatant were measured and expressed as μmole tyrosine/g muscle.

2.7. Determination of α-amino acids

The method of Benjakul and Morrissey (1997) was used to measure α-amino acids. The muscle (3 g) was homogenised with 30 ml of 5% SDS (85 °C) and incubated at 85 °C for 30 min to allow the protein to be solubilised. An aliquot supernatant (125 μl) was mixed with 2.0 ml of 0.213 M phosphate buffer, pH 8.2. One ml of 0.01% TNBS solution was added, mixed thoroughly and incubated at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulfite and the mixture was cooled at room temperature (25 °C) for 15 min. The absorbance was measured at 420 nm and α-amino acids were expressed as L-leucine.

2.8. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

The protein pattern of lizardfish muscle was analysed by SDS–PAGE, according to the method of Laemmli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85 °C, were added to the sample (3 g). The mixture was then homogenised using homogeniser (IKA Labortechnik, Malaysia) for 2 min. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins. The samples were centrifuged at 3500×*g* for 20 min to remove undissolved debris. Protein concentration was determined according to the method of Lowry, Rosebrough, Farr, and Randall (1951) using bovine serum albumin as standard. SDS–PAGE gel was made of 10% running gel and 4% stacking gel. After separation, the proteins were fixed and stained with Coomassie Blue R-250.

2.9. Surimi and surimi gel preparation

Fish kept in ice at different times were washed with tap water. The flesh was removed manually and minced to uniformity. The mince was then washed with cold water (5 °C) at a mince/water ratio of 1:2 (w/w). The mixture was stirred gently for 3 min and washed mince was filtered with a layer of nylon screen. The washing process was repeated twice. Finally, the washed mince was subjected to centrifugation using a model CE 21 K basket centrifuge (Grandiumpiant, Belluno, Italy) with a speed of 700×*g* for 15 min. The washed mince was kept in ice until used.

Surimi prepared was treated with 2.5% salt and the moisture content was adjusted to 80%. The mixture was chopped for 5 min at 4 °C to obtain the homogeneous sol. The sol was then stuffed into polyvinylidine casings with a diameter of 2.5 cm and both ends of casings were sealed tightly. One-step heated gels were prepared by heating the sol at 90 °C for 20 min. Two-step heated gels were prepared by incubating the sol at 40 °C for 30 min or 25 °C for 3 h, followed by heating at 90 °C for 20 min. The gels were cooled in iced water and stored for 24 h at 4 °C prior to analysis.

2.10. Texture analysis

Texture analysis of surimi gels was carried out using a texture analyser TA-XT2 (Stable Micro System, Surrey, UK). Gels were equilibrated and evaluated at room temperature (28–30 °C). Five cylinder-shaped samples, with a length of 2.5 cm, were prepared and subjected to determination. Breaking force (gel strength) and deformation (elasticity/deformability) were measured using the texture analyser equipped with a cylindrical plunger (diameter 5 mm; depression speed 60 mm/min).

2.11. Determination of whiteness

Surimi gel colour was determined using a JP7100F colorimeter (Juki Corporation, Tokyo, Japan). L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were measured and whiteness was calculated as described by Fujii, Watanabe, and Maruyama (1973) as follows:

$$\text{Whiteness} = 100 - \left[(100 - L^*)^2 + a^{*2} + b^{*2}\right]^{1/2}$$

2.12. Statistical analysis

Analysis of variance (ANOVA) was performed and mean comparisons were obtained by Duncan's multiple range test (Steel & Torrie, 1980)

3. Results and discussion

3.1. Changes in TVB and TMA

Changes in TVB and TMA content of lizardfish during iced storage are shown in Fig 1. The values increased as the storage time increased ($P < 0.05$). At the same storage period, TVB and TMA contents of headed/eviscerated samples were generally lower than those of whole fish ($P < 0.05$). The initial TVB content of 10.01 mg/100 g in fish samples at day 0 suggested that fish muscle possibly underwent some deterioration. TVB content in albocore was reported to increase with storage time (Perez-Villarreal & Pozo, 1990). At day 0, TMA was also found at a level of 1.07 mg/100 g, indicating some extent of trimethylamine oxide (TMAO) reduction. Throughout the iced storage of 15 days, TMA content of whole samples was approximately 1.5–2.3-fold higher than that of headed/eviscerated samples. Both TVB and TMA contents in the whole samples increased at a higher rate than those of the headed/eviscerated samples ($P < 0.05$). Viscera and gills are the major sources of enzymes, as well as microorganisms. Therefore, the removal of these organs presumably resulted in a lower hydrolysis of the nitrogenous compounds. The formation of TVB and TMA is generally associated with growth of microorganisms and can be used as an indicator of spoilage. However, TVB content was not a good index of quality of black-skipjack during iced storage (Mazorra-Manzano, Pacheco-Aguilar, Diaz-Rojas, & Lugo-Sanchez, 2000). Specific spoilage bacteria such as *Shewanella putrefaciens*, *Photobacterium phosphoreum*, and *Vibrionaceae*, typically use TMAO as an electron acceptor in anaerobic respiration, resulting in off-odour and off-flavour due to the formation of TMA (Gram & Huss, 1996; Huss, 1995). The TVB and TMA formed in fish during iced storage were probably mediated by psychrotropic bacteria (Sasajima, 1973, 1974). The results suggest that pretreatment of lizardfish by heading and eviscerating effectively retarded the spoilage during iced storage for up to 15 days. TVB of 25 mg/100 g was suggested to be a limit level for sardine (Marrakchi, Bennour, Bouchriti, Hamama, & Tagafait, 1990), while a TVB value of 30–40 mg/100 g was reported to be the limit for acceptability of cold and temperate water fish (Connell, 1975). Additionally, Bennour, Marrakchi, Bouchart, Hamama, and Ousdda (1991) found a TMA of 5 mg/100 g as the rejection value for mackerel.

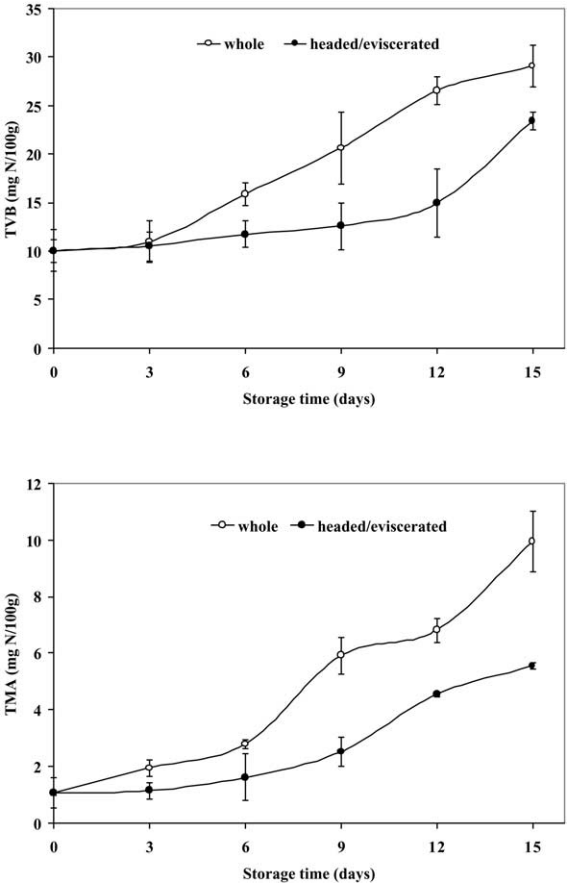

Fig. 1. TVB and TMA contents in whole and headed/eviscerated lizardfish during 15 days of iced storage. Bars indicate the standard deviation from triplicate determinations.

3.2. Changes in TCA-soluble peptides, α-amino acids and protein pattern

TCA-soluble peptides and α-amino acids in both whole and headed/eviscerated lizardfish increased throughout 15 days of iced storage ($P < 0.05$), suggesting the autolytic degradation of fish protein (Fig. 2). Generally, whole fish had more TCA-soluble peptides than headed/eviscerated fish, especially when the storage time increased. At days 12 and 15, TCA-soluble peptides in whole samples were 1.61- and 1.31-fold of those obtained in headed/eviscerated samples. Higher α-amino acids were also found in whole fish, compared to headed/eviscerated fish (Fig. 2). The cleavage of peptide bond can be monitored by α-amino acid content. The results indicated that hydrolysis of peptide bonds occurred during iced storage. TCA-soluble peptide and α-amino acid contents observed in all samples were conversely proportional, with myosin heavy chain remaining in the sample (Fig. 3).

SDS–PAGE patterns revealed that myosin heavy chain was much more susceptible to hydrolysis than was actin. This result was in agreement with Benjakul et al. (1997) who reported that myosin heavy chain was more prone to proteolytic degradation than other muscle proteins, for example actin, troponin, and tropomyosin. From the results, it was found that pretreatment of lizardfish, by heading and eviscerating, effectively prevented the degradation of myosin heavy chain. Viscera contain a variety of digestive proteinases, which play a role in the softening of abdominal tissues during post-

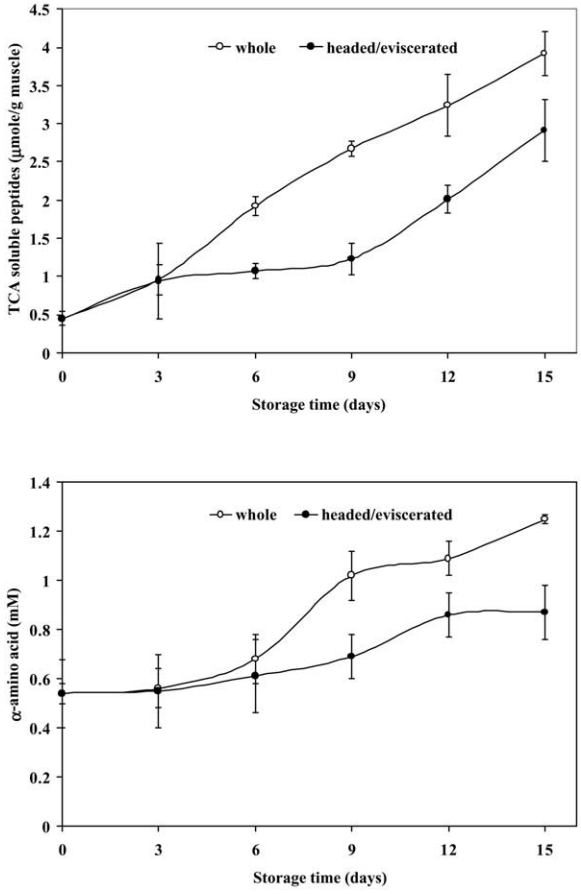

Fig. 2. TCA-soluble peptides and α-amino acids in whole and headed/eviscerated lizardfish during 15 days of iced storage.

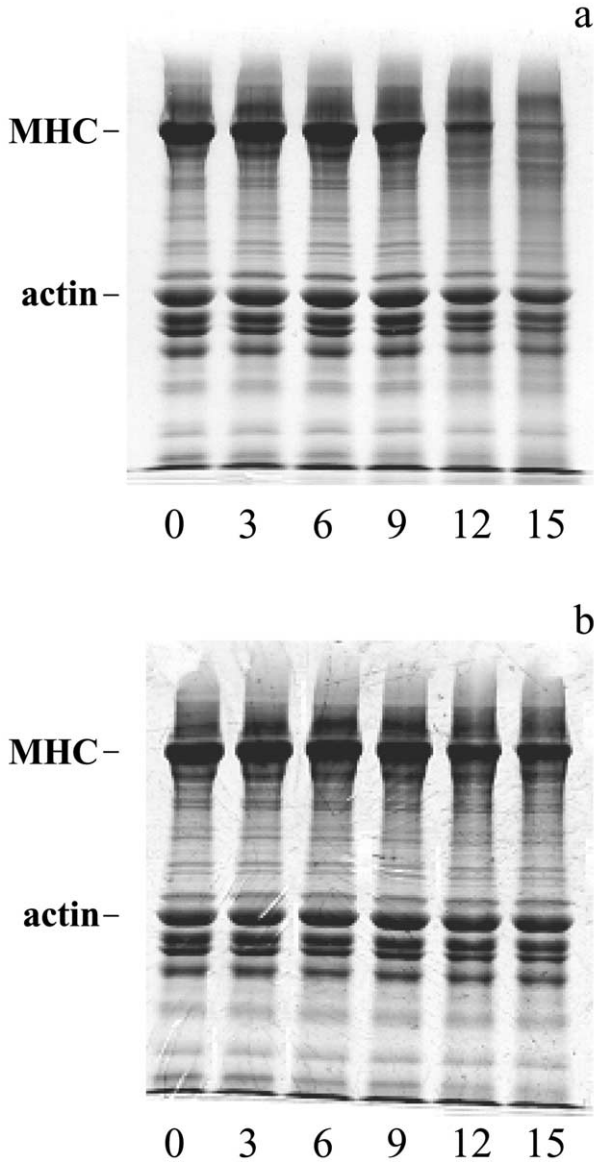

Fig. 3. SDS–PAGE pattern of muscle proteins from whole (a) and headed/eviscerated (b) lizardfish during 15 days of iced storage. Numbers designate storage time (days).

mortem storage of fish (Haard, 1994). Therefore, eviscerating resulted in the reduction of digestive enzymes, leading to more retained myosin heavy chain. Apart from digestive proteinases, viscera contain a number of bacteria, which expedite the spoilage of fish. Furthermore, muscle proteinases, such as cathepsins and calpain, also play an essential role in protein degradation of fish muscle (Haard, 1994). This was reconfirmed by the observation of increase in both TCA-soluble peptides and α-amino acids in headed/eviscerated samples. However, degradation of muscle protein, caused by microbial proteinase, was also presumed. Three species of lizardfish used for surimi production in western Japan contained the heat-activated proteinases, which potentially hydrolysed the muscle protein at the elevated temperature ranging from 40 to 70 °C (Suwansakornkul et al., 1993). High proteinase activity in the spawning period was closely associated with the low gel-forming ability of lizardfish (*Saurida elongata*) (Shimizu & Wendakoon, 1990). Although those endogenous muscle proteinases in lizardfish were active at high temperature, they were able to hydrolyse the muscle protein in combination of microbial proteinases during the extended iced storage. Thus, pretreatment of lizardfish by heading and eviscerating could retard the degradation of muscle protein.

3.3. Changes in formaldehyde

It was notable that formaldehyde content increased continuously as the storage time increased ($P < 0.05$; Fig. 4). However, formaldehyde formation in whole samples was approximately two-fold higher than that of headed/eviscerate samples. The result indicated that heading and eviscerating had a profound effect on the reduction of formaldehyde formation in lizardfish. Formaldehyde is formed by demethylation of TMAO, a compound present in most marine species. This phenomenon is common for gadoid species (Hebard, Flick,

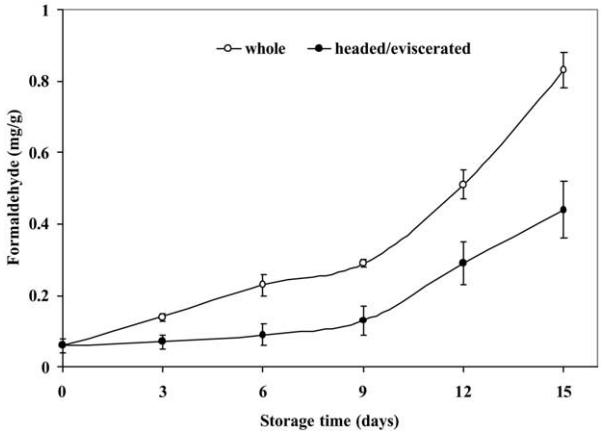

Fig. 4. Formaldehyde content in whole and headed/eviscerated lizardfish during 15 days of iced storage.

& Martin, 1982) and occurs during frozen storage, causing an alteration in the characteristics of fish muscle protein, particularly changes in functional properties (Careche, Cofrades, Carballo, & Colmenero, 1998). Formaldehyde accelerated the formation of high-molecular weight polymer in cod myosin (Ang & Hultin, 1989; Careche & Li-Chan, 1997). The formation of formaldehyhe was evident in lizardfish, indicating that lizardfish had high contents of both TMAO and TMAO demethylase. It was noted that headed/eviscerated fish contained much lower amounts of formaldehyde. Visceral organs are known to be the most active in the formation of formaldehyde. Harada (1975) reported the enzymatic formation of DMA and formaldehyde in the muscle, viscera and liver. Among those samples, liver of lizardfish (*S. tumbil*) had the greatest ability to produce formaldehyde (Harada, 1975). Moreover, Dingle and Hines (1975) found that kidney tissue was responsible for formation of formaldehyde in mince of gadoid fish. From the results, headed/eviscerated lizardfish also contained formaldehydyde. Kimura, Seki, and Kimura (2000) found TMAO demethylase in walleye pollack myofibrillar fraction, which causes the reduction of TMAO to dimethylamine and formaldehyde. Therefore, the formation of formaldehyde in lizardfish stored in ice can be impeded by removal of viscera and heads. However, formaldehyde was still formed at a lower rate in the muscle. Thus, heading and eviscerating may retain the functionality of muscle protein, especially the gelation property, during the prolonged iced storage.

3.4. Changes in ATPase activity

ATPase activities were monitored in both whole and headed/eviscerated lizardfish during 15 days of iced storage (Fig. 5). A slight decrease in Ca^{2+}-ATPase activity of lizardfish natural actomyosin was observed throughout iced storage. Slightly higher decreasing rate was observed in whole fish than in headed/eviscerated

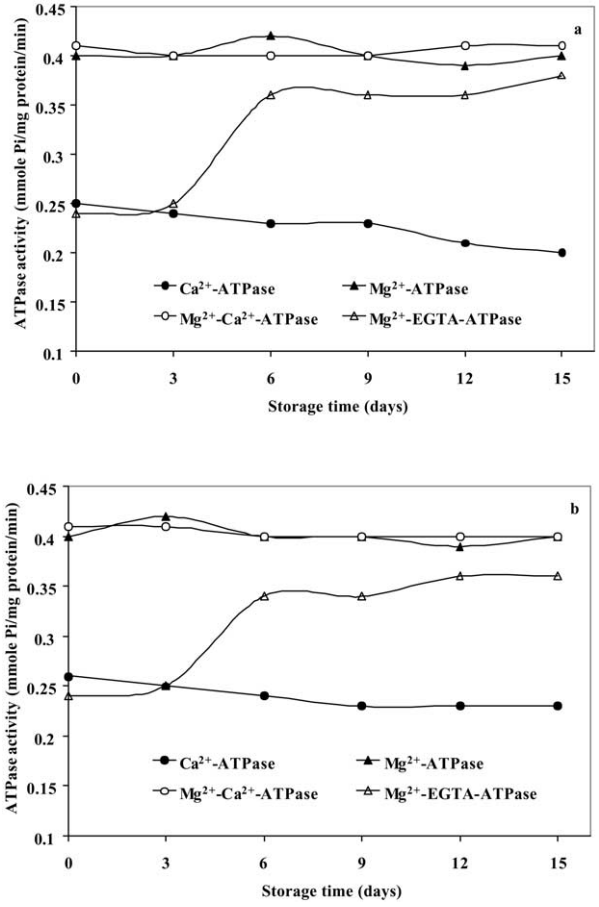

Fig. 5. ATPase activities of natural actomyosin from whole (a) and headed/eviscerated (b) lizardfish during 15 days of iced storage.

fish. The results indicated that myosin underwent some changes in native conformation during iced storage. Ca^{2+}-ATPase has been used as an indicator of myosin integrity (Benjakul et al., 1997). The loss in Ca^{2+}-ATPase was possibly associated with the proteolysis of myosin molecule (Ouali & Valin, 1981). Ca^{2+}-ATPase of ordinary muscle of sardine, during 6 days iced storage, decreased to 50% with concomitant degradation of MHC due to proteolysis (Seki, Oogane, & Watanabe, 1980). Moreover, denaturation of myosin was possibly caused by the oxidation of SH groups or disulfide interchanges as well as changes in surface hydrophobicity during iced storage (Benjakul et al., 1997). From the results, no changes in either Mg^{2+}-ATPase or Mg^{2+}–Ca^{2+}-ATPase activities were observed in both whole and headed/eviscerated samples throughout 15 days of iced storage (Fig. 5). The result suggested no changes in actin-myosin complexes in the presence of both endogenous and exogenous calcium ions (Benjakul et al., 1997). Nevertheless, marked increase in Mg^{2+}–EGTA-ATPase activity was found as the storage time increased ($P<0.05$), indicating loss in troponin–tropomyosin complexes (Benjakul et al., 1997; Ouali & Valin, 1981). The changes in troponin C, a calcium binding

protein, were possibly associated with the loss of Ca^{2+} sensitivity. Ca^{2+} sensitivity is a good indicator of Ca^{2+} regulation in myofibrillar proteins (Roura & Crupin, 1995). The decrease in Ca^{2+} sensitivity was observed after 3 days of iced storage (Fig. 6). Lower Ca^{2+} sensitivity was found in whole fish, than in headed/eviscerated fish after 9 days and a marked difference was observed at day 15. Benjakul et al. (1997) postulated that decrease in Ca^{2+} sensitivity in Pacific whiting actomyosin during iced storage was caused by proteolysis. Therefore, the decrease in Ca^{2+} sensitivity in lizardfish probably resulted from the denaturation or degradation of troponin, especially troponin C. From the results, heading and eviscerating of lizardfish could retard the denaturation of myosin and troponin during iced storage, compared to whole fish.

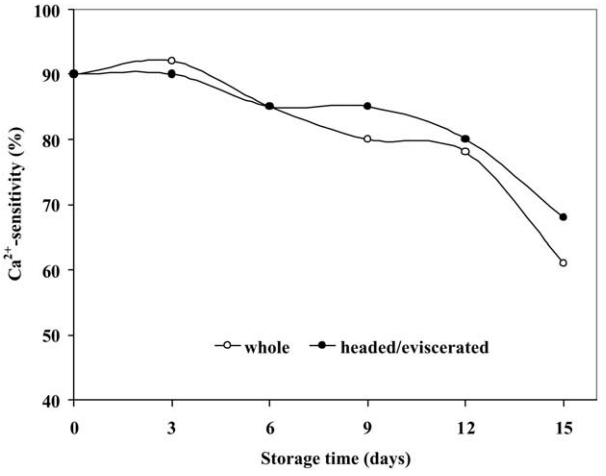

Fig. 6. Ca^{2+}-sensitivity of natural actomyosin from whole and headed/eviscerated lizardfish during 15 days of iced storage.

3.5. Changes in surimi gel-forming ability

Breaking force and deformation of surimi gels prepared from whole and headed/eviscerated lizardfish stored in ice are shown in Fig. 7. Different heating conditions for gel preparation caused different gelling properties. Two-steps, heating with high temperature (40 °C) setting prior to heating at 90 °C, showed the highest breaking force and deformation ($P<0.05$), which exhibited a twice higher breaking force than directly heated gel. Gel prepared by medium temperature (25 °C) setting, followed by heating at 90 °C had a lower breaking force and deformation than high temperature set gel but higher values than directly heated gel (90 °C). This result indicates that setting played an important role in cross-linking of gel network, especially by non-disulfide covalent bonds induced by endogenous transglutaminase. Transglutaminase was found to mediate the formation of myosin cross-linking via the ε-(γ-glutamyl-lysine) linkage (Seki et al., 1990). When comparing high and medium temperature setting, superiority of the former gel to the latter was observed. This was probably because endogenous transglutaminase in

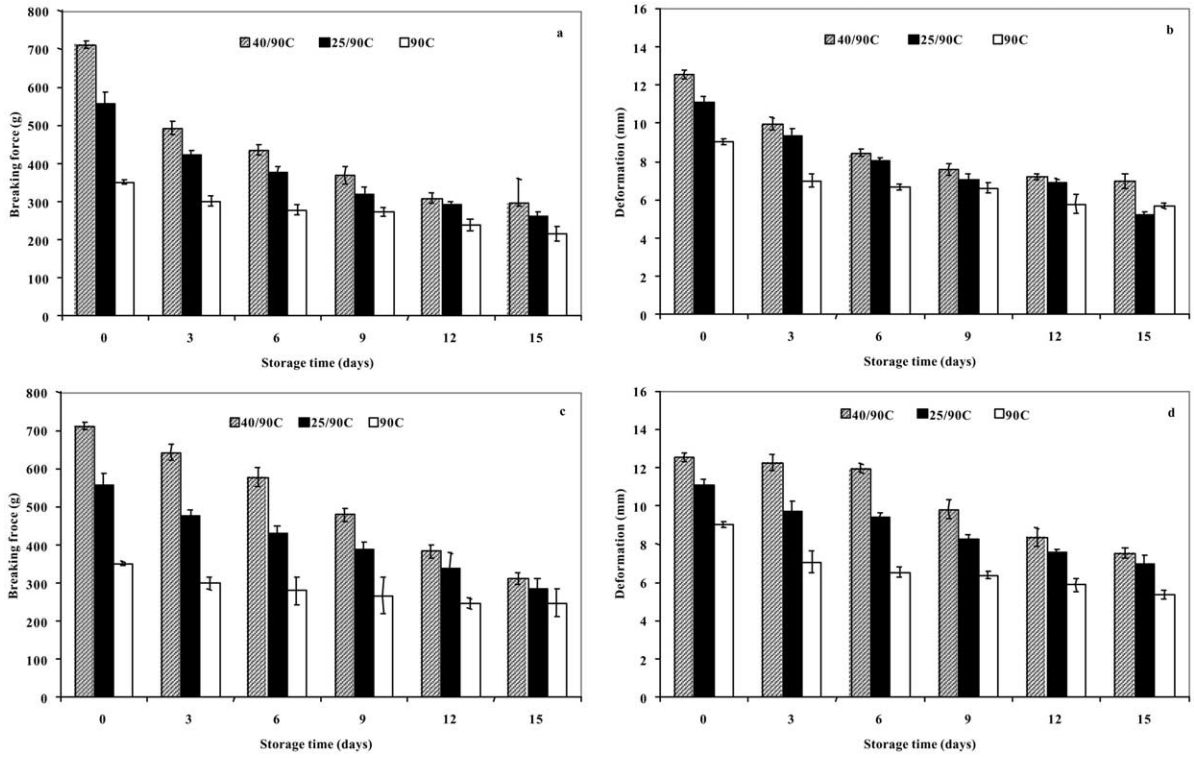

Fig. 7. Breaking force and deformation of surimi gels from whole (a and b) and headed/eviscerated (c and d) lizardfish during 15 days of iced storage. The gels were prepared under different setting and /or heating conditions.

lizardfish muscle was much more active at 40 °C, than at 25 °C. Furthermore, the alignment or aggregation of protein probably occurred to a higher extent at higher temperature. At sufficiently high temperature, both hydrophobic interaction and disulfide bond formation could occur (Niwa, 1992). Thereafter, unfolded proteins underwent more aggregation, via both disulfide and hydrophobic interactions between molecules, as the temperature was elevated to 90 °C. From the results, setting of lizardfish surimi sol prior to heating is crucial for resulting gel quality.

When the storage time increased, breaking force of surimi produced, from both whole and headed/eviscerated fish, decreased up to 15 days of storage ($P<0.05$). Surimi gel prepared with different heating conditions showed the same trend. However, heading and eviscerating was effective in retaining the gel forming ability during iced storage. At day 3, breaking force of surimi gel from whole fish, prepared by high temperature setting, followed by heating, was decreased to 69%, while that from headed/eviscerated fish was decreased to only 90%. Deformation was decreased to 79 and 98% for the gel from whole and headed/eviscerated fish, respectively. However, no marked differences were found between surimi gel from whole and headed/eviscerated fish, prepared by direct heating. It was postulated that heading and eviscerating were able to maintain the transglutaminase activty, which functioned as a gel enhancer during the setting process. Transglutaminase in whole lizardfish was probably inactivated to a higher extent, than in headed/eviscerated samples. This was postulated to be caused by higher proteinase released and formaldehyde formed in whole samples during iced storage, leading to the inactivation of transglutaminase. The decrease in gel–forming ability of lizardfish surimi protein was concomitant with the increase in formaldehyde, TCA-soluble peptides and α-amino acids, as well as the decrease in myosin heavy chain content. Myosin integrity is of paramount importance for gelation (An et al., 1996). The degradation of myosin resulted in an inferior gel network formation, causing a lower elasticity with poor water-holding capacity in the gel matrix. Additionally, formaldehyde formed in lizardfish, particularly for the whole fish, was able to bind to some groups in protein side chains and form intra and intermolecular methylene bridges (Sikorski, Kolakowska, & Burt, 1990). These reactions induced protein aggregation and decreased protein solubility. Therefore, formaldehyde was important in protein denaturation, resulting in a lower gel forming ability (Careche & Li-Chan, 1997). The addition of formaldehyde to hake natural actomyosin caused a reduction in gel strength (Careche et al., 1998).

Continuous decreases in breaking force and deformation were observed when storage time increased ($P<0.05$). This was coincidental with the increase in protein denaturation and degradation with increasing storage time. The rate of decreases in both breaking force and deformation were higher in whole fish than headed/eviscerated fish. Kurokawa (1979) reported that gel strength of kamaboko made from lizardfish stored in ice for 3 days was less than 50% of that made from fresh fish. Yean (1993) also found a decrease in gel strength of surimi produced from threadfin bream stored in ice for more than 2 days. Chalmers, Careche, and Mackie (1992) reported a decrease in gel strength of cod natural actomyosin after 6 days of iced storage. Therefore, storage time was a prime factor determining the gel quality of lizardfish. Nevertheless, heading and eviscerating of lizardfish could be a promising means to maintain the gel-forming ability of muscle protein during iced storage by reduction of protein degradation and denaturation.

Gel whiteness markedly decreased as storage time increased ($P<0.05$; Fig. 8). Higher decreasing rate of whiteness was found in surimi gel produced from whole fish, compared to headed/eviscerated fish. During iced storage, oxidation of pigments in fish muscle, particularly myoglobin or hemoglobin, occurred. These oxidised products possibly bound tightly with muscle

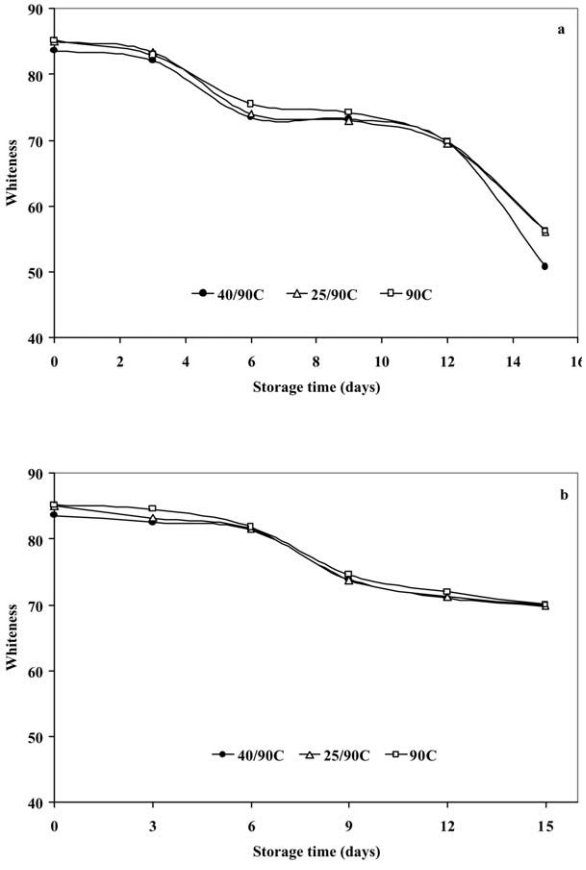

Fig. 8. Whiteness of surimi gels from whole (a) and headed/eviscerated (b) lizardfish during 15 days of iced storage.

proteins, especially in the presence of formaldehyde and could not be removed by washing. As a consequence, surimi gel produced from fish kept for a longer time had lower whiteness. Whiteness of surimi gel from headed/eviscerated fish had higher whiteness than those produced from whole fish. Marked differences were found when the storage time increased. During extended storage, blood and liquid from internal organs in whole samples could penetrate through the muscle, especially when autolysis proceeded and caused a looser muscle structure. No differences in whiteness were observed among gel samples prepared under the different heating processes. Therefore, both pretreatment and storage time directly affected the whiteness of surimi gels from lizardfish.

4. Conclusion

Lizardfish rapidly underwent physico-chemical changes by both denaturation and proteolytic degradation during iced storage, leading to loss in gel-forming ability. With extended storage time, the poor gelling characteristics of lizardfish were mainly caused by degradation of myosin heavy chain, as well as by formaldehyde formation. The heading and eviscerating of fish, prior to iced storage, effectively maintain the properties of muscle proteins, resulting in better surimi gel quality.

Acknowledgements

Authors would like to thank Prince of Songkla University for the financial support.

References

An, H., Peters, M. Y., & Seymour, T. A. (1996). Roles of endogenous enzymes in surimi gelation. *Trends in Food Science and Technology*, *7*, 321–327.

Ang, J. F., & Hultin, H. O. (1989). Denaturation of cod myosin during freezing after modification with formaldehyde. *Journal of Food Science*, *54*, 814–818.

Amano, K., Yamada, K., & Bito, M. (1963). Detection and identification of formaldehyde in gadoid fish. *Bulletin of the Japanese Society of Scientific Fisheries*, *29*, 695–701.

Benjakul, S., & Morrissey, M. T. (1997). Protein hydrolysates from Pacific whiting solid wastes. *Journal of Agricultural and Food Chemistry*, *45*, 3423–3430.

Benjakul, S., Seymour, T. S., Morrissey, M. T., & An, H. (1997). Physicochemical changes in Pacific whiting muscle proteins during iced storage. *Journal of Food Science*, *62*, 729–733.

Benjakul, S., Visessangaun, W., Riebroy, S., Ishizaki, S., & Tanaka, M. Gel-forming properties of two species of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*, as affected by pretreatment and iced storage. *Journal of the Science of Food and Agriculture* (in press).

Bennour, M., Marrakchi, A. E., Bouchart, N., Hamama, A., & Ousdda, M. E. (1991). Chemical and microbiological assessments of mackerel (*Scomber scombrus*) stored in ice. *Journal of Food Protection*, *54*, 784 789–792.

Careche, M., & Li-Chan, E. (1997). Structural changes and raman spectroscopic studies of cod myosin upon modification with formaldehyde or frozen storage. *Journal of Food Science*, *62*, 717–723.

Careche, M., Cofrades, S., Carballo, J., & Colmenero, F. J. (1998). Emulsifying and gelation properties during freezing and frozen storage of hake, pork, and chicken actomyosins as affected by addition of formaldehyde. *Journal of Agricultural and Food Chemistry*, *46*, 813–819.

Chalmers, M., Careche, M., & Mackie, I. M. (1992). Properties of actomyosin isolated from cod (*Gadus morhua*) after various periods of storage in ice. *Journal of the Science of Food and Agriculture*, *58*, 375–383.

Connell, J. J. (1975). *Control of fish quality*. Surrey: Fishing News.

Conway, E. J., & Byrne, A. (1936). An absorbtion apparatus for the micro-determination of certain volatile substances. I. The micro-determination of ammonia. *Biochemistry Journal*, *27*, 419–429.

Dingle, J. R., & Hines, J. A. (1975). Protein instability in minced flesh from fillets and frames of several commercial Atlantic fishes during storage at −5 °C. *Journal of Fisheries Research Board of Canada*, *32*, 775–783.

Fiske, C. H., & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry*, *66*, 375–400.

Fujii, Y., Watanabe, K., & Maruyama, Y. (1973). Relation between the ATP-breakdown in ice-stored Alaska pollack meat and the quality of frozen surimi. *Bull Tokai Reg Fish. Res Lab*, *75*, 7–11.

Gram, L., & Huss, H. H. (1996). Microbial spoilage of fish and fish products. *International Journal of Food Microbiology*, *33*, 121–137.

Haard, N. F. (1994). Protein hydrolysis in seafoods. In F. Shahidi, & J. R. Botta (Eds.), *Seafoods: chemistry, processing technology and quality* (pp. 10–33). New York, USA: Chapman & Hall.

Harada, K. (1975). Studies on enzyme catalyzing the formation of formaldehyde and dimethylamine in tissues of fishes and shellfishs. *Journal of Shimonoseki University of Fisheries*, *18*, 296–302.

Hebard, C. E., Flick, G. J., & Martin, R. E. (1982). Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In R. E. Martin, G. J. Flick, & D. R. Ward (Eds.), *Chemistry and biochemistry of marine food products* (pp. 149–304). Connecticut, USA: AVI Publishing Company.

Huss, H. H. (1995). *Quality and quality changes in fresh fish*. FAO fisheries technical paper no. 348.

Kimura, M., Seki, N., & Kimura, I. (2000). Occurrence and some properties of trimethylamine-N-oxide demethylase in myofibrillar fraction from walleye pollack muscle. *Fisheries Science*, *66*, 725–729.

Kurokawa, T. (1979). Kamaboko-forming ability of frozen and ice stored lizard fish. *Bulletin of the Japanese Society of Scientific Fisheries*, *45*, 1551–1555.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature*, *227*, 680–685.

Lin, D., & Morrissey, M. T. (1995). Northern squawfish (Ptychocheilus oregonensis) for surimi production. *Journal of Food Science*, *60*, 1245–1247.

Lowry, Q. H., Rosebrough, N. J., Farr, L. A., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 256–275.

MacDonald, G. A., Lelievre, J., & Wilson, N. D. C. (1990). The strength of gels prepared from washed and unwashed minces of hoki (*Macrerinus novaezelandiae*) stored in ice. *Journal of Food Science*, *55*, 976–978.

Marrakchi, E., Bennour, A. M., Bouchriti, N., Hamama, A., & Tagafait, H. (1990). Sensory, chemical and microbiological assessments of Moroccan sardines (*Sardina pilchardus*) stored in ice. *Journal of Food Protection*, *53*, 600–605.

Mazorra-Manzano, M. A., Pacheco-Aguilar, R., Diaz-Rojas, E. I., &

Lugo-Sanchez, M. E. (2000). Postmortem changes in black skipjack muscle during storage in ice. *Journal of Food Science*, *65*, 774–779.

Morrissey, M. T., Wu, J. W., Lin, D. D., & An, H. (1993). Protease inhibitor effects on torsion measurements and autolysis of Pacific whiting surimi. *Journal of Food Science*, *58*, 1050–1054.

Morrissey, M. T., & Tan, S. M. (2000). World resources for surimi. In J. W. Park (Ed.), *Surimi and surimi seafood* (pp. 1–21). New York, USA: Marcel Dekker.

Niwa, E. (1992). Chemistry of surimi gelation. In T. C. lanier, & C. M. Lee (Eds.), *Surimi technology* (pp. 389–427). New York, USA: Marcel Dekker.

Ouali, A., & Valin, C. (1981). Effect of muscle lysosomal enzymes and calcium activated neutral proteinase on myofibriallar ATPase activity: relationship with aging changes. *Meat Science*, *5*, 233–245.

Perez-Villarreal, B., & Pozo, R. (1990). Chemical composition and iced spoilage of albacore (*Thunnus alalunga*). *Journal of Food Science*, *55*, 678–682.

Roura, S. I., & Crupin, M. (1995). Biochemical and functional properties of myofibrils from pre and post-spawned hake (*Merluccius hubbi Martini*) stored in ice. *Journal of Food Science*, *60*, 269–272.

Sasajima, M. (1973). Studies on the psychrotelerant bacteria in fish and shellfish. IV. Relation between the number of trimethylamine oxide-reducing psychrotrophic bacteria and their activity. *Bulletin of the Japanese Society of Scientific Fisheries*, *39*, 511–518.

Sasajima, M. (1975). Studies on psychrotolerant bacteria in fish and shellfish. V. The growth or viability of trimethylamine oxide-reducing psychrotrophic bacteria and their activity at subzero temperatures. *Bulletin of the Japanese Society of Scientific Fisheries*, *40*, 630–635.

Seki, N., Oogane, Y., & Watanabe, T. (1980). Changes in ATPase activities and other properties of sardine myofibrillar protein during ice-storage. *Bulletin of the Japanese Society of Scientific Fisheries*, *46*, 607–615.

Seki, N., Uno, H., Lee, N., Kimura, I., Toyoda, K., Fujita, T., & Arai, K. (1990). Transglutaminase activity in Alaska pollack muscle and surimi and its reaction with myosin B. *Nippon Suisan Gakkaishi*, *56*, 125–132.

Shimizu, Y., & Kaguri, A. (1986). Influence of death condition and freshness on the gel-forming property of fish. *Bulletin of the Japanese Society of Scientific Fisheries*, *52*, 1837–1841.

Sikorski, Z., Kolakowska, A., & Burt, J. R. (1990). Postharvest biochemical and microbial changes. In Z. E. Sikorski (Ed.), *Seafood: resources, nutritional composition and preservation* (pp. 55–75). Florida, USA: CRC Press.

Shimizu, Y., & Wendakoon, C. N. (1990). Effects of maturation and spawning on the gel-forming ability of lizardfish (*Saurida elongata*) muscle tissues. *Journal of the Science of Food and Agriculture*, *52*, 331–338.

Shimizu, Y., Machida, R., & Takenami, S. (1981). Species variations in gel-forming characteristics of fish meat paste. *Nippon Suisan Gakkaishi*, *47*, 95–104.

Suwansakornkul, P., Itoh, Y., Hara, S., & Obatake, A. (1993). The gel-forming characteristics of lizardfish. *Nippon Suisan Gakkaishi*, *59*, 1029–1037.

Steel, R. G. D., & Torrie, J. H. (1980). *Principles and procedures of statistics: a biometrical approach* (2nd ed). New York: McGraw-Hill.

Yasui, A., & Lim, P. Y. (1987). Changes in chemical and physical properties of lizardfish meat during ice and frozen storage. *Nippon Shokuhin Kyogo Gakkaishi*, *34*, 54–60.

Yean, Y. S. (1993). The quality of surimi made from threadfin bream stored on ice for different periods. *International Journal of Food Science and Technology*, *28*, 343–346.