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Original Article

Contribution of the polymerization of protein by disulfide bonding to increased gel strength of walleye pollack surimi gel with preheating time

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ABSTRACT: To clarify the contribution of polymerization of myosin heavy chain (MHC) by disulfide bonding to increased gel strength of cooked gel via preheating, the pastes of walleye pollack surimi (SS and C grades) were preheated at 25°C and 40°C for a variety of hours prior to heating at 80°C for 20 min. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) patterns of cooked gels were analyzed with and without reducing the samples, which were solubilized in 8 M urea–2% SDS solution. The formation of polymers by disulfide bonding in cooked gels was almost constant in each of the SS and C grade surimi gels despite the period of preheating. Therefore, it was suggested that polymerization by disulfide bonding occurred during cooking at 80°C and not during preheating.

KEY WORDS: **disulfide bond, gel formation, myosin heavy chain, suwari, walleye pollack surimi.**

INTRODUCTION

Gelled elastic fish meat product, kamaboko, is a very popular fish product in Japan. One of its important qualities is elasticity, which is called 'ashi'. To make an elastic and cohesive gel from salt-ground surimi, a preheating process below 40°C prior to cooking at 80°C or 90°C is very important. Gel formation during preheating is called 'suwari' or setting, and preheating at temperatures between 25°C and 40°C are most effective for walleye pollack surimi.[1] Setting is known to be attended by the cross-linking of myosin heavy chains (MHC) that is mediated by transglutaminase (TGase).[2–6]

Another form of covalent bonding; that is, intermolecular disulfide bonding through the oxidation of sulfhydryl groups, in carp actomyosin has been suggested to be involved in gel formation.[7,8,9] Furthermore, it was found that disulfide bonding is involved in the polymerization of MHC during the heating of carp and flying fish actomyosin in temperatures exceeding 30–40°C.[10] Other studies indicate that the sulfhydryl group content of walleye pollack surimi remains constant during incubation at 38°C,[11] and that intermolecular disulfide bonding is thought to be indispensable to the setting appearance of some species of fish meat.[12] Furthermore, it was reported that SH-blocked actomyosin gel was weaker in gel strength than SH-unblocked actomyosin gel at 40°C under conditions of no TGase activity.[13]

However, it is not clear whether the increase in the gel strength of the final cooked gel that has undergone prolonged preheating is related to the formation of disulfide bonds through the oxidation of sulfhydryl groups other than cross-linking by TGase.

The present study was conducted to clarify the contribution of MHC polymerization by intermolecular disulfide bonding to increased gel strength of final cooked gel as well as its contribution to the setting of two different grades of walleye pollack surimi pastes.

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MATERIALS AND METHODS

Materials

Unsalted SS grade (Maruha Co Ltd, Tokyo, Japan) and C grade (Hirose Suisan Co. Ltd, Hokkaido, Japan) walleye pollack frozen surimi were used. Moisture and protein contents were, respectively, 76% and 16% (SS grade surimi) and 78% and 14.5% (C grade surimi).

Chemical composition analysis

Moisture content was determined by air-drying a given sample in an infra-red moisture determination balance (FD-600-2; Kett Electric Laboratory, Tokyo, Japan). Protein content was determined using the Kjeldhal method.

Gel preparation from surimi

Unsalted walleye pollack surimi was kept overnight at 4°C for thawing. Each surimi was adjusted to 80% moisture content and ground together with 3% NaCl for 20 min. The resulting pastes were stuffed into stainless steel cylinder cases (3.1 cm in diameter and 3.0 cm in height), wrapped in polyvinylidene chloride film and then heated in a water bath at various temperatures (20–80°C) for 20 min and 2 h. In another case, pastes were also preincubated at 25°C and 40°C for several hours (0–5 h) before heating at 80°C for 20 min. The gels were then cooled immediately in ice water and stored at 4°C until required for assessment of gel properties.

Gel strength measurement

Before measuring gel strength, samples were held at room temperature for 2 h. Gel strength (g/cm^2) was assessed by multiplying breaking strength (g/cm^2) by elongation ($\Delta L/L$, ΔL breaking length; L, sample length, usually 1 cm), which were measured by a rheometer (Model CR-200D; Sun Scientific Co. Ltd, Tokyo, Japan) according to the method of Shimizu *et al.*[14] For each treatment 10 determinations were performed and the mean values and standard deviation were calculated.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

A small piece of heated gel (0.4 g) was homogenized in a Teflon homogenizer with 18 mL of 0.05 M sodium phosphate buffer (pH 7.2) containing 8 M urea–2% SDS and 2 mL of 7.2 mM *N*-methylmaleimide (NEM), and then heated for 2 min in boiling water. After homogenizing again the next day, to make unreduced samples, 2 mL of the obtained sample solution was mixed with an equal volume of 50% glycerol–0.4% SDS–0.075 M phosphate buffer (pH 7.2) and 0.05% bromophenol blue. To make reduced samples, 2 mL of sample solution was mixed with an equal volume of 20% 2-mercaptoethanol in 50% glycerol–0.4% SDS–0.075 M phosphate buffer and 0.05% bromophenol blue (pH 7.2). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Weber and Osborn[15] using 3% polyacrylamide gel in a vertical disc-gel system (8.0 cm length, 5 mm diameter). Then, 10 μL of the mixture was applied to each disc gel. Disc gels were stained with Coomassie Brilliant Blue R 250.

Densitometry of stained disc gel

Stained disc gels were scanned at a wavelength of 640 nm using a chromatoscanner (Dual-wavelength Flying-spot scanner, S-9000; Shimadzu, Kyoto, Japan). To check the behavior of MHC upon heating we estimated: (i) the total density of polymers, whose bands appeared above MHC, as an index of polymerization of MHC; (ii) the total density of the substances, whose bands appeared between MHC and actin, as an index of degradation of MHC; and (iii) the density of MHC.

RESULTS AND DISCUSSION

Temperature–gelation curves of high- and low-grade walleye pollack surimi

In order to confirm the gel-forming characteristics of high and low grades of walleye pollack surimi (SS and C grade), their salted surimis were heated at 20–80°C at 10°C intervals for 20 min and 2 h. Gel strength is shown in Fig. 1. SS grade and C grade surimi gels showed similar temperature–gelation patterns. When heated for 20 min, maximum gel strength was demonstrated at 40°C in both surimis. Maximum gel strength was demonstrated at 30°C for 2 h of heating. The gel strength of SS grade surimi set at 30°C for 2 h was approximately 2.5 times higher than that of C grade surimi. At temperatures above 40°C, gel strength decreased with prolonged heating time (from 20 min to 2 h). At 50–60°C for 2 h, gel strength was the weakest over a range of heating temperatures, indicating that

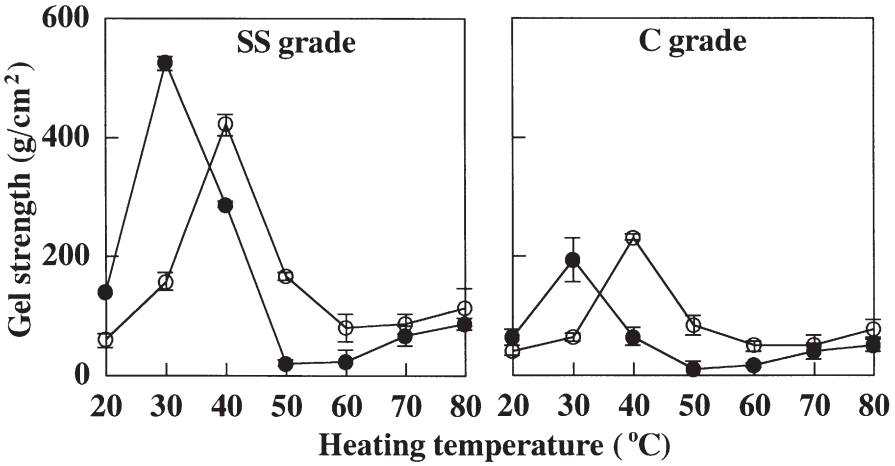

Fig. 1 Changes in gel strength of SS and C grade walleye pollack surimi heated at various temperatures for (○) 20 min and (●) 2 h. Vertical bars represent the standard deviation.

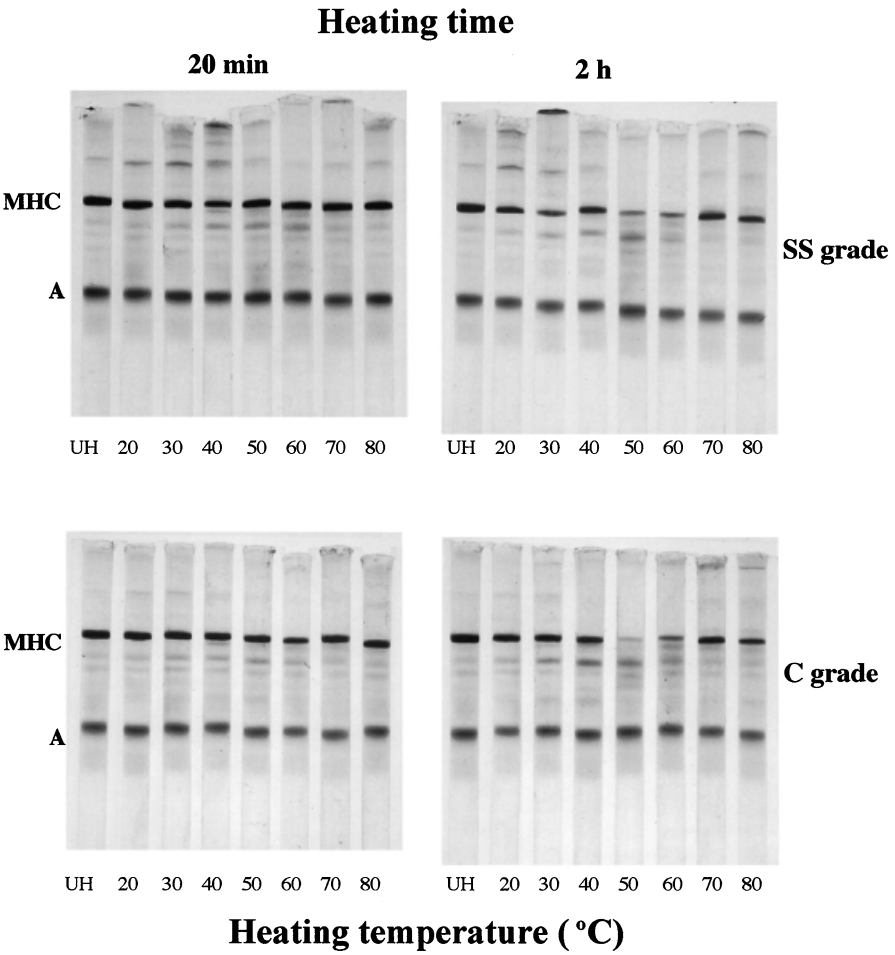

Fig. 2 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) patterns of reduced samples of walleye pollack surimi (SS and C grade) gels heated at various temperatures for 20 min and 2 h. UH, unheated surimi paste; MHC, myosin heavy chain; A, actin.

modori was strongest at approximately these temperatures in both surimis.

The temperature–gelation curves of SS grade and C grade surimi showed similar patterns to that of SA grade walleye pollack surimi reported by Shimizu *et al.*[14] Furthermore, the time dependence of gel formation at various temperatures was also similar to that of SA grade surimi reported by Numakura *et al.*[16] Therefore, it was confirmed that the gel-forming characteristics of surimi used in the present experiment are similar to those of surimi reported previously.

SDS-PAGE patterns of the reduced samples of each gel are shown in Fig. 2. In SS grade surimi, the formation of a polymer, whose band appeared above that of MHC on the electrophoresis gels, was clearly observed up to 40°C for 20 min-heated gels, and up to 30°C for 2 h-heated gels along with a decrease of MHC. This means that the polymerization of MHC occurred up to 30–40°C. The polymerization of MHC in C grade was not as strong as that in SS grade surimi; SS grade surimi appears to have stronger polymerization activity of MHC compared with C grade. The temperature at which the strongest setting and polymerization of MHC were observed for the two types of walleye pollack surimi is in agreement with results reported by Nishimoto *et al.*[1] and Numakura *et al.*[16] Furthermore, in both grades of surimi, it was observed that substances (MHC-A) whose bands appeared between the MHC band and the actin band on SDS-PAGE, were formed between 30°C and 60°C

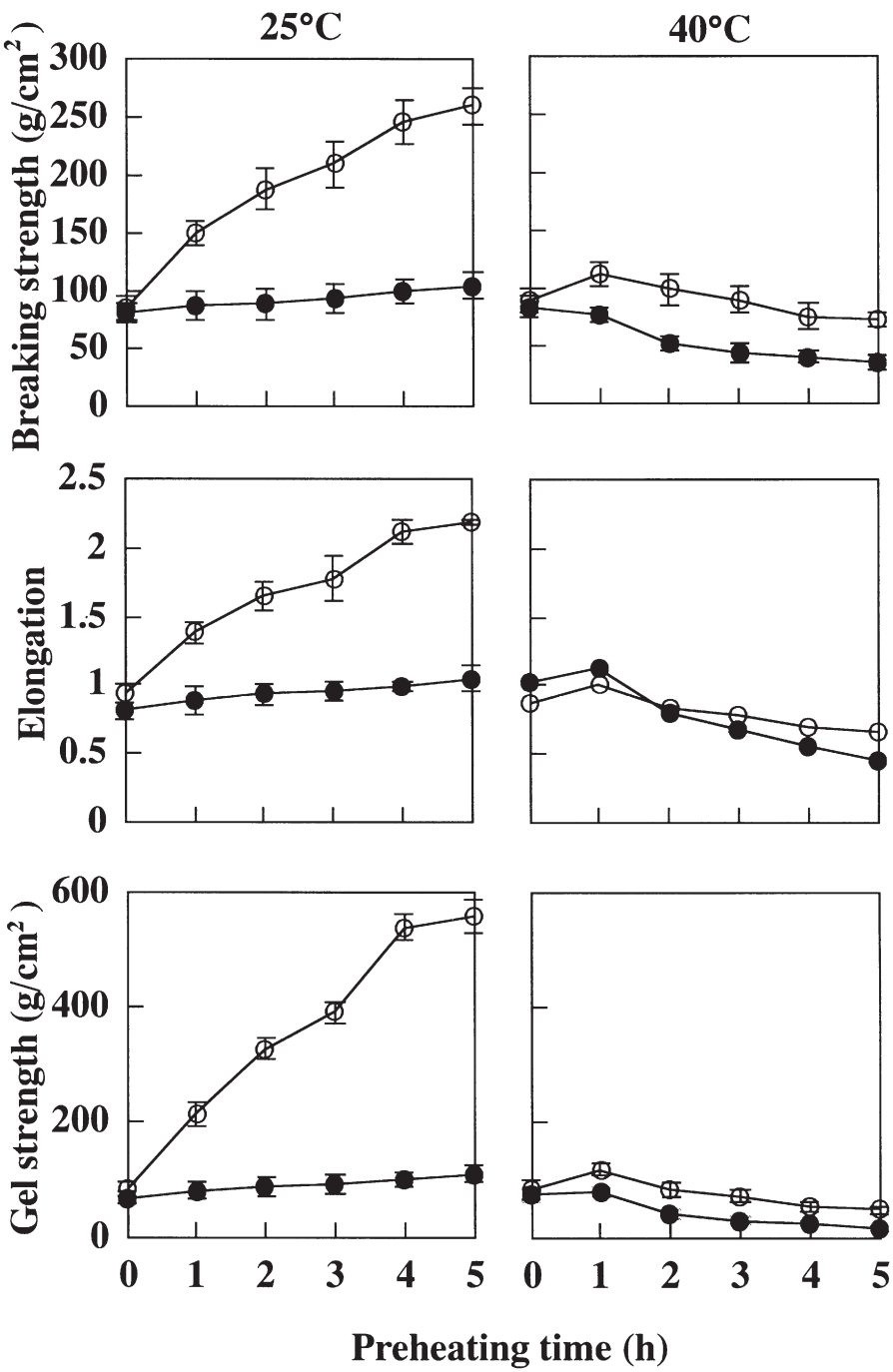

Fig. 3 Changes in breaking strength, elongation and gel strength of (○) SS and (●) C grade walleye pollack surimi gel during preheating at 25°C and 40°C for various periods before being heated at 80°C for 20 min. Vertical bars represent the standard deviation.

and was accompanied by a decrease in MHC, especially in 2 h-heated gels. This means that the degradation of MHC occurred. The degradation of MHC appears to occur more strongly in C grade surimi gels than in SS grade surimi gels. The most probable candidates for causing the degradation of MHC at approximately 50°C is a serine protease and a cysteine protease.[6,17,18]

From this observation it was reconfirmed that suwari occurs strongly at around 30°C and is accompanied by the polymerization of MHC through non-disulfide bonding, and that modori occurs at around 50°C and is accompanied by the degradation of MHC in walleye pollack surimi paste regardless of its grade. In addition, it was indicated that MHC polymerization was notably stronger in SS grade surimi, whereas MHC degradation was stronger in C grade surimi.

Effect of preheating at 25°C and 40°C on gel strength of cooked gel

In order to determine the contribution of disulfide bonding, as well as non-disulfide covalent bonding, and the degradation of MHC during preheating on setting to the gel strength of cooked gel, pastes prepared from SS and C grade surimi were preheated at before cooking at 80°C. The preheating temperatures of 25°C and 40°C were used for the low temperature setting and high temperature setting, respectively.

Behaviors of breaking strength, elongation and gel strength of cooked gels are shown in Fig. 3. When preheating occurred at 25°C, the values of these properties increased with increasing preheating time in the same manner for each surimi gel. After 5 h preheating, gel strength of SS grade surimi gel was approximately 600 g/cm^2, whereas that of C grade surimi gel was approximately 100 g/cm^2. These results indicate that the setting effect is stronger in SS grade surimi than in C grade surimi, as reported previously.[19–23] At 40°C, breaking strength, elongation and gel strength increased slightly up to 1 h and then decreased gradually for SS grade surimi gel, whereas these characteristics decreased after 1 h for C grade surimi gel and showed no increases at all. These results demonstrate that at 40°C the response of paste from walleye pollack surimi to setting was positive initially but preheating beyond 1 h resulted in a weakening of the gels. After 5 h preheating, the gel strength of SS grade surimi gel was 47 g/cm^2, whereas that of C grade surimi gel was 16 g/cm^2. In other words, these results suggest that modori is slightly stronger in C grade than in SS grade surimi gel. The behavior of gel strength during preheating, which was evaluated by a stretching test of SS and C grade surimi gels, were almost similar to the results of Abe *et al.*,[19,20] who evaluated various grades of walleye pollack surimi using a penetration method. It has been reported that the difference between high- and low-grade walleye pollack surimi in their gel-forming ability is related to the polymerization ability of MHC by non-disulfide covalent bonding during setting.[21–23]

In order to estimate the amount of polymerization that occurred through disulfide bonding in the

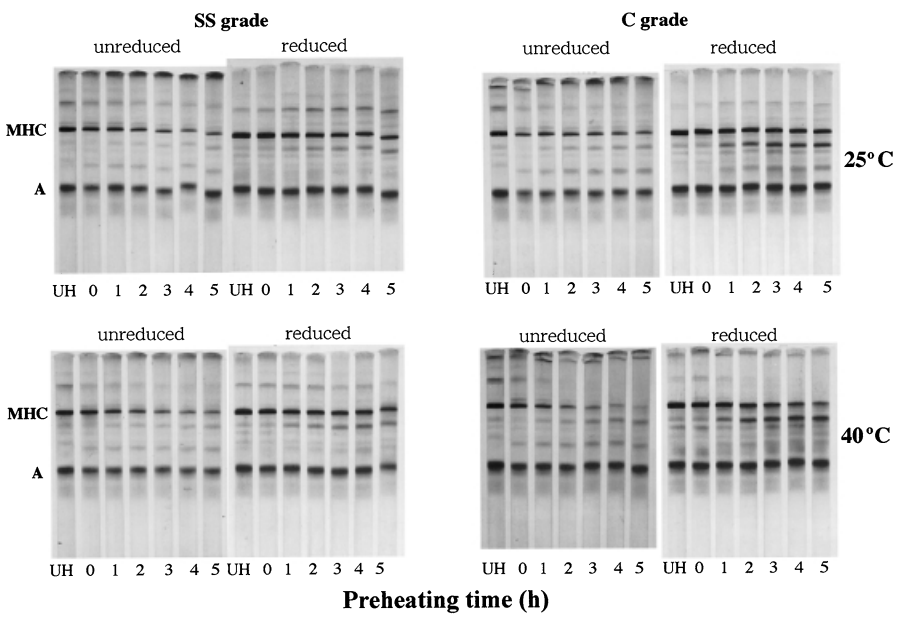

Fig. 4 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) patterns of reduced and unreduced samples of walleye pollack surimi (SS and C grade) gels preheated at 25°C and 40°C for various periods before being heated at 80°C for 20 min. UH, unheated surimi paste; MHC, myosin heavy chain; A, actin.

cooked gels of SS and C grade surimi, SDS-PAGE patterns of unreduced and reduced samples were analyzed. The patterns are shown in Fig. 4 and the densitometric data are shown in Fig. 5 (at 25°C) and Fig. 6 (at 40°C). It was clear that at 25°C, polymers (i.e. molecules larger in size whose bands appeared above MHC) in unreduced samples increased but MHC decreased as preheating was prolonged. Actin remained almost constant during preheating, indicating that the major changes resulted from a decrease in the amount of MHC. In unreduced samples of SS grade surimi gels, MHC decreased proportionally to the increase in polymer and the slight increase in substances between myosin heavy chain and actin (MHC-A) as preheating time was prolonged. In contrast, in unreduced samples of C grade surimi gels, polymer formation was weaker than in those of SS grade surimi gels, and a greater amount of MHC-A was formed in the former compared with the latter.

In reduced samples, the amount of polymers formed is less than that in unreduced samples of

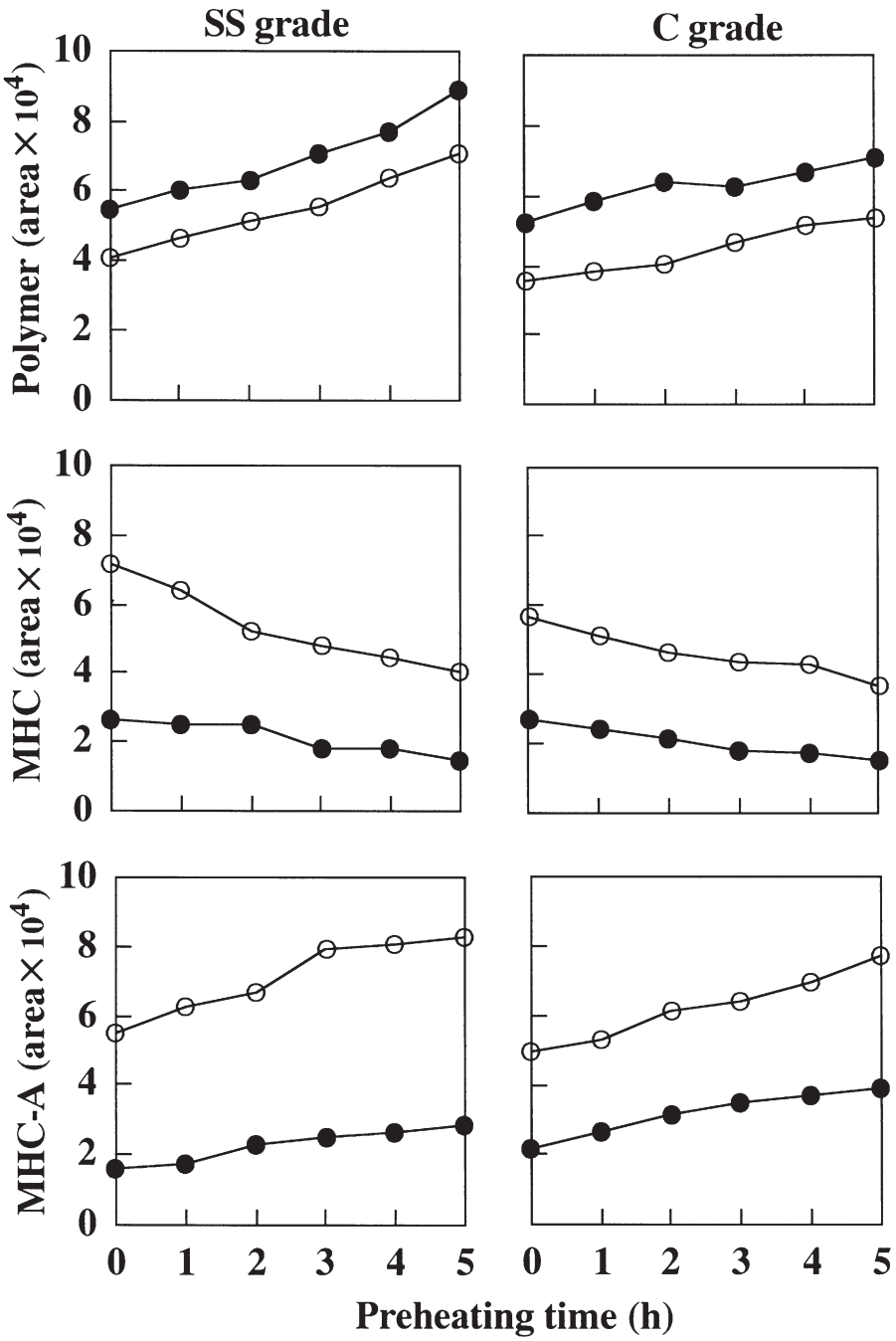

Fig. 5 Staining density of protein subunits in SS and C grade walleye pollack surimi gels preheated at 25°C for various periods before being heated at 80°C for 20 min. (○) Reduced samples; (●) unreduced samples. Polymer, molecules larger than myosin heavy chain (MHC) in size; MHC-A, substances that are between MHC and actin in size.

both grades of surimi gels. However, the amount increased almost proportionally to that in unreduced samples during the preheating. This means that the contribution of intermolecular disulfide bonding was almost constant and that disulfide bonding was not formed during preheating at 25°C but was formed mainly during the second heating at 80°C. In addition, the amount of MHC-A substances increased after reducing the samples. This indicates that the degraded MHC substances were oxidized into bigger molecules. In other words, degradation and oxidation might occur independently.

At 40°C, polymerization of MHC was observed initially in unreduced samples in both SS and C grade surimi gels, but preheating longer than 1 h resulted in a decrease in amount of polymer. The difference between unreduced and reduced samples at 25°C and 40°C in the contents of polymer, MHC, and MHC-A were not affected by the preheating temperature, similar to the difference between unreduced and reduced samples at 25°C. This also indicated that disulfide bonding did not proceed during preheating at 40°C, but occurred mainly during the second heating at 80°C.

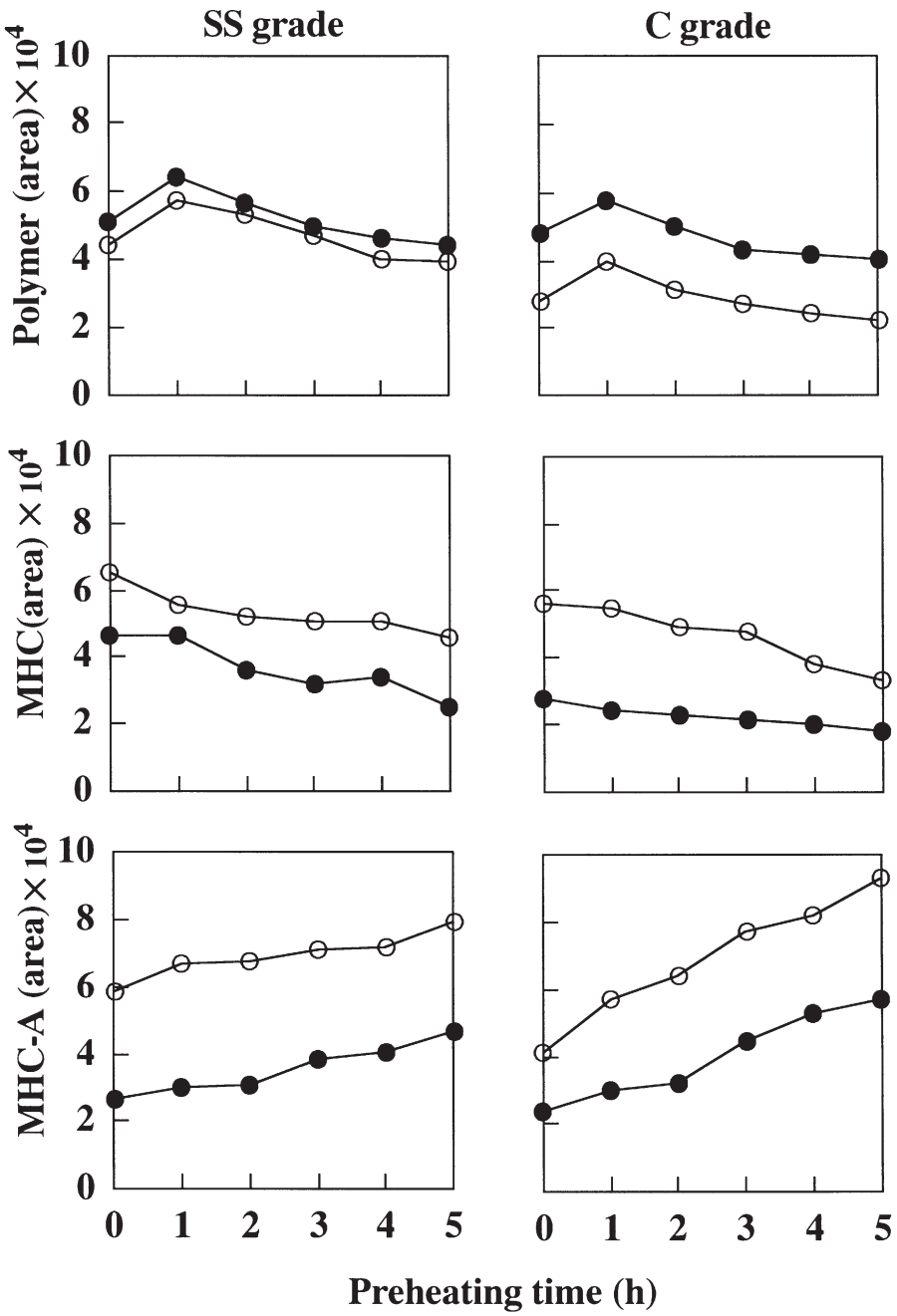

Fig. 6 Staining density of protein subunits in SS and C grade walleye pollack surimi gels preheated at 40°C for various periods before being heated at 80°C for 20 min. (○) Reduced samples; (●) unreduced samples.

Previously, Runglerdkriangkrai *et al.* reported that MHC polymers are formed through disulfide bonding between S-1 accompanied by the exposure of sulfhydryl groups at temperatures above 30°C.[24] Results of the presents study are not consistent with their report. The difference might be because of the presence of sugar in surimi, which increases the heat stability of ATPase activity.[25] This means that the S-1 portion is more stable in the presence of sugar and that it is more difficult to expose and oxidize the sulfhydryl groups in S-1.

From the results of the present study, the formation of polymers by disulfide bonding in cooked gels was almost constant in each of the SS grade and C grade surimi gels despite the period of preheating at 25°C and 40°C. Therefore, it is suggested that the polymerization by disulfide bonding occurs during cooking at 80°C and not during preheating

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