Studies on the Oxidation Behavior during Washing Process of Fish Meat

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Abstract : To investigate the oxidation of SH groups in the washing process of fish meat, washed fish meats from four factories in Kochi prefecture were sampled in the winter and the summer seasons. SH content, SDS-PAGE and ATPase activities were analyzed. SH content of all samples in the winter season were higher values than in the summer season. The same patterns in SDS-PAGE were observed in the winter as well as in the summer season. Dimer of myosin heavy chain were observed in all samples and cannot be reduced with mercaptoethanol. The results from the ATPase activities indicated that not only SH_1 and SH_2 on myosin head portion, but also SH_a on the LMM, did not oxidize during the washing process. Therefore, it was clearly observed that the oxidation of fish meat through the formation of disulfide bonds did not occur strongly during washing process in the surimi-based products manufacturing of Kochi prefecture.

Keywords : oxidation of SH groups, washed meat fish, ATPase activities

Introduction

Myofibrillar protein plays an important role on the gel forming ability of surimi-based products. Especially, both myosin and actomyosin have dominant roles in surimi gelation.[1-3] Water washing of fish meat is a necessary process in surimi manufacturings to remove sarcoplasmic protein and refine myofibrillar protein. The washing process will upgrade the ashi forming ability and will inhibit freeze denaturation of protein.[4] However, we found that the formation of disulfide bonds through the oxidation of SH groups in the fish meat before grinding results in reducing gel forming ability.[5]

To investigate whether the oxidation of SH groups in fish meat occur in the washing step of the surimi-based products manufacturing or not, washed fish meat were sampled from four big factories of surimi-based products in Kochi prefecture in the winter and the summer seasons. The oxidation behavior of fish meat was examined by measuring the total SH content as well as the SDS-polyacrylamide gel electrophoresis (SDS-PAGE). To evaluate the denaturation of myosin as well as myofibrillar protein, various ATPase activities of myofibril were measured.

Materials and Methods

Materials Washed fish meats were sampled from the surimi-based product factories in Kochi prefecture during winter (January-March, 2002) and summer seasons (July-September, 2002).

Preparation of Myofibril Myofibril was prepared from washed meat according to the method of Katoh et al.[6] Five grams of washed meat was homogenized with 15 ml of 0.1 M KCl-40 mM borate buffer (pH 7.0) for 1 min at 20,000 rpm. The same buffer (85 ml) was added and centrifuged at 2,400 rpm for 10 min. The pellet was suspended in 20 ml of the same buffer and centrifuged at 5,000 rpm for 10 min. The suspension and centrifugation were repeated for 2 more times. The pellet was collected and the protein concentration of myofibril was adjusted to 4 mg/ml in the same buffer. The resulting solution was used for measurement of ATPase activities.

Measurement of Total SH Content The total SH group content was determined using 5,5'-dithiobis 2-nitrobenzoic acid, (DTNB) by the method of Ellman.[7] Washed meat (0.1g) was solubilized in 8 M Urea - 2% SDS - 10 mM EDTA in 0.2 M Tris-HCl buffer (pH6.8). To 4 ml of the mixture, 0.4 ml of 0.1% DTNB solution was added and incubated at 40°C for 25 min. After incubation, absorbance of the mixture was measured on a Hitachi U-1000 spectrophotometer at 412 nm. The SH group content was calculated from the absorbance using the molar extinction coefficient of 13,600 M^{-1} cm^{-1} for 2-nitro-5-thiobenzoic acid at this wavelength.

SDS-PAGE The washed meat (0.1g) was solubilized with 20ml of 8 M Urea - 2% SDS - 50 mM phosphate buffer (pH6.8) containing 2.5mM *N*-ethylmaleimide (NEM) using a Teflon homogenizer. For the reduced samples, the homogenates were mixed with 10% volumes of 2-mercaptoethanol. Unreduced samples were prepared by adding deionized water instead of 2-mercaptoethanol. Samples (20 μl) were applied to 3% acrylamide gel, and SDS-PAGE was carried out according to the method of Weber and Osborn.[8]

Measurement of ATPase Activities The measurement of ATPase activities was carried out according to the method of Seki and Narita.[9]

Results and Discussion

Changes in Total SH Content It is well known that sulfhydryl groups and disulfide bonds are important in maintaining structure and functional properties of native protein. Moreover, the thiol groups and disulfide bonds also play an important role on thermal gelation and freeze denaturation.[10-12] In addition, disulfide bonds are related to the gel strength of the fish meat.[5] The water used for meat washing is a tap water that is not a pure water as used in laboratory. Therefore, it was supposed that the SH groups are susceptible to be oxidized during the washing processing. Therefore, the SH content was measured to observe the oxidation behavior. The SH content in the winter season varied from 8.4 to 8.76.mol/10^5g protein whereas from 7.93 to 8.43 in the summer. From these results, it was clearly appeared that the fish meat in the winter had higher SH content than in the summer (Fig. 1). However, the SH content of all samples were about 8 mol/10^5g protein. This suggests that the oxidation is not so strong.

Changes in SDS-PAGE To clarify whether the polymerization by disulfide bonds exists in the washed meat or not, SDS-PAGE was conducted both in the unreduced and the reduced samples of the washed

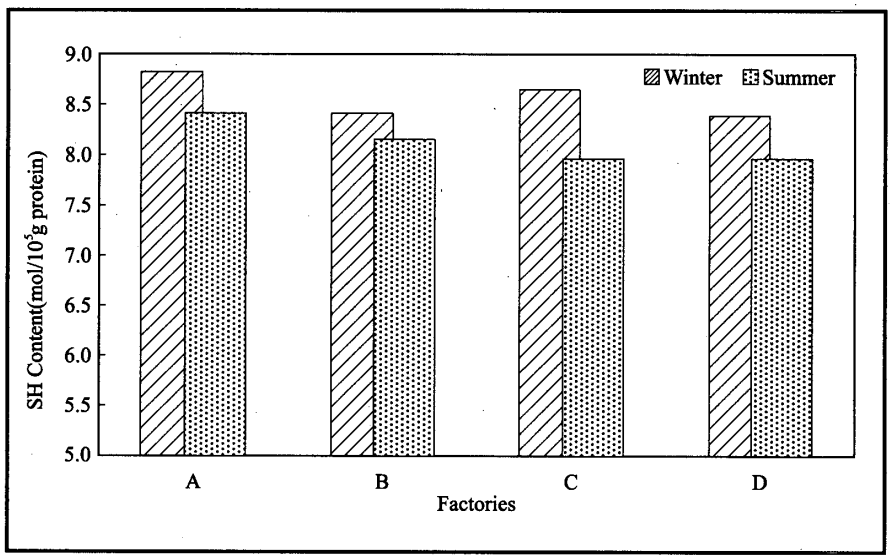

Fig. 1. Changes in SH content of washed meat in the winter and summer season.

meats. The almost same patterns in SDS-PAGE were observed in the unreduced and the reduced samples either in the winter or in the summer samples (Fig. 2 and 3). However, the light bands at the dimer position of myosin heavy chain were observed in the unreduced samples. In the reduced samples, these bands were lighter in the samples of factory A and C but still observed in the dimer position. In addition, there were the other light bands on the top of acrylamide gel (at polymer position) in all samples that were not reduced with mercaptoethanol. However, the polymerlization level in the unreduced samples varied with the factories. The sample from factory C showed the darker dimer and polymer bands than the other samples. Moreover, it appeared the darker dimer and polymer bands in summer sample than in the winter one.

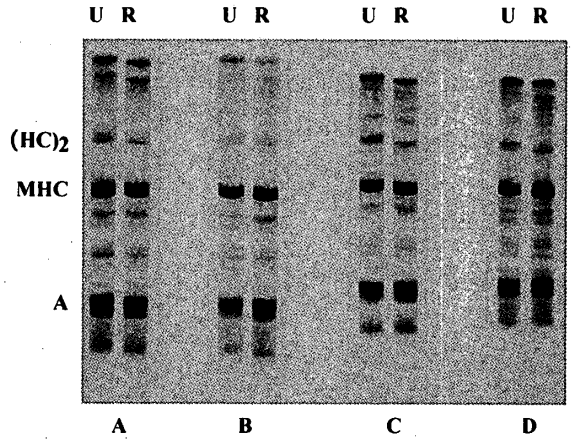

Fig.2. SDS-PAGE patterns of washed meat from four factories in the winter season.

U = Unreduced sample,

R = Reduced sample

$(HC)_2$ = Dimer of myosin heavy chain

MHC = Myosin heavy chain

A = Actin

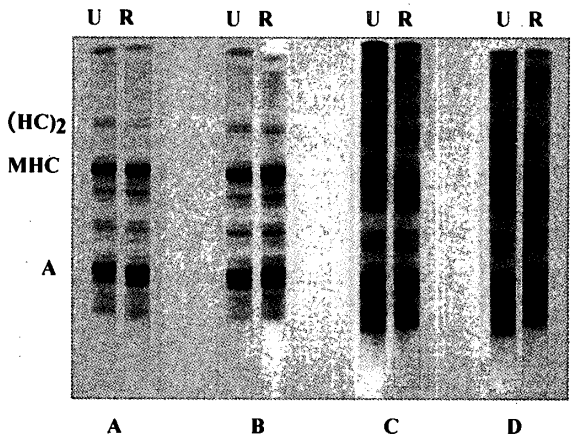

Fig.3. SDS-PAGE patterns of washed meat from four factories in the summer season. The label is the same as described in Fig. 2.

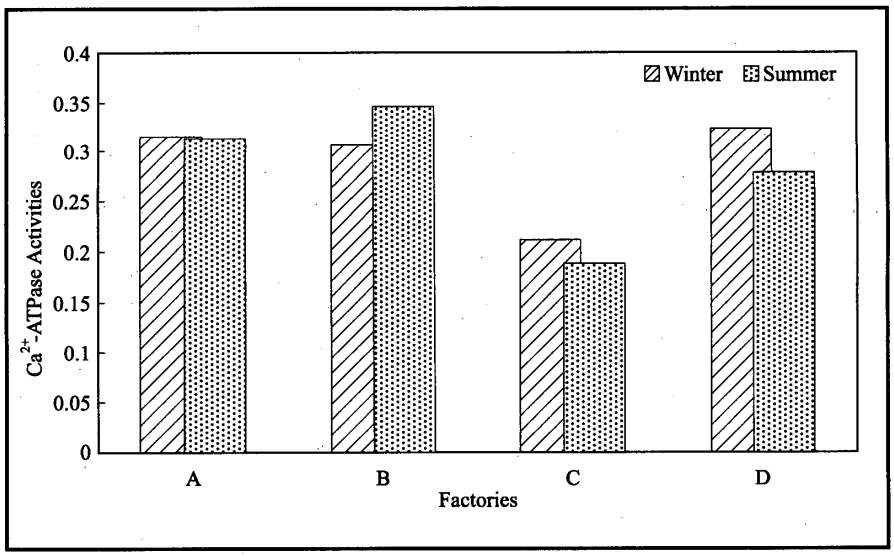

Fig. 4. Changes in Ca^{2+} -ATPase activities of washed meat in the winter and the summer seasons.

From SDS-PAGE, the reduced samples showed the same patterns as the unreduced samples. Therefore, this polymerization is not due to disulfide bonds but might be due to isopeptide bonds activated by trans glutaminase (TGase). It was clarified that polymerization by SS bonding is not so strong during washing process.

Changes in ATPase Activities To examine the denaturation of myosin as well as myofibrillar protein in washed meat, various ATPase activities were measured. Two different kinds of SH groups, termed SH_1 and SH_2 have been reported to be found on the myosin head portion and are involved in ATPase activities of myosin.[13-16] Blocking of the SH_1 with NEM results in an activation of the Ca^{2+} -ATPase with a concomitant loss in the EDTA(K^+)-ATPase.[15] Blocking of both sulfhydryls, SH_1 and SH_2, eliminates both ATPase activities.[16] To evaluate whether the reactive SH groups on the myosin head portion were already oxidized or not, Ca^{2+} -ATPase and EDTA ATPase activity were measured. The washed meat from three factories (A, B and D) showed the high value in the Ca^{2+} -ATPase activities both in the winter and the summer seasons (Fig. 4). The EDTA-ATPase activities showed the similar trend as the Ca^{2+} -ATPase activities (Fig. 5). These results indicated that the SH_1 and SH_2 on the myosin head portion were not oxidized during the washing process. Since the Ca^{2+} -ATPase activity is the denaturation index of myosin head portion[4], the myosin head portion from three factories in the winter season were more in the native state than that of factory C which had lower value in the ATPase activities both in the winter and the summer seasons. In addition, these washed meat from three factories could make the stronger gel products than the washed meat from factory C.

Yamashita et al.[17-19] found another SH group, termed SH_a, which was localized in the light meromyosin (LMM) region of myosin molecule and was responsible for Mg^{2+} -ATPase activity. The characteristic of SH_a in the washed meat was also observed in this study by measuring Mg^{2+} (Ca^{2+})-ATPase and Mg^{2+} -(EGTA)-ATPase activities. The Mg^{2+} (Ca^{2+})-ATPase in the winter season showed the almost same activities from four factories and higher activities than those in the summer season, except for factory B (Fig. 6). However, the Mg^{2+} -(EGTA)-ATPase from factory A and B in the summer season showed the higher activities than those in the winter season, in contrast with the almost

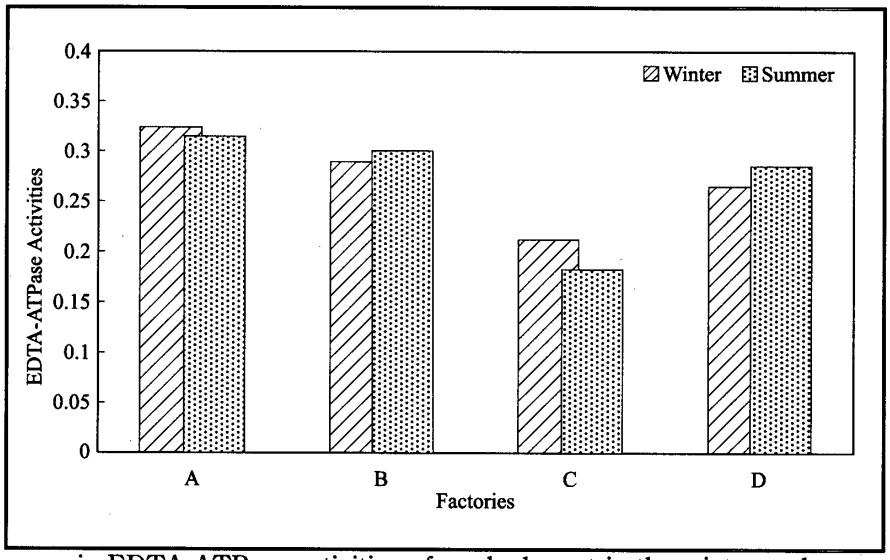

Fig. 5. Changes in EDTA-ATPase activities of washed meat in the winter and summer season.

same activities of the samples from factory C and D in the winter and the summer seasons (Fig. 7). The high activity in both Mg^{2+} -ATPase showed that the SH_a on the LMM portion of myosin was not oxidized during the washing process yet.

Ca^{2+} -sensitivity (which is a denaturation index of tromponin and trompomyosin) was calculated from Mg^{2+} (Ca^{2+})-ATPase and Mg^{2+} (EGTA)-ATPase activities. It was clearly observed that the Ca^{2+} -sensitivities, like the Mg^{2+} (Ca^{2+})-ATPase activities, showed higher values in the winter than those in the summer season (Fig. 8). It was demonstrated that the tromponin and trompomyosin of the fish meat in the summer season were more denatured than the washed meat in the winter season. It was reported that the myofibrillar ATPase total activities of surimi was closely related to the jelly strength of kamaboko from the same material.[6] Therefore, the high value in ATPase activities from factory A, B and D were suggested to make the stronger gel than the lower value in ATPase activities from factory C.

The results from the ATPase activities indicated that not only SH_1 and SH_2 on myosin head por-

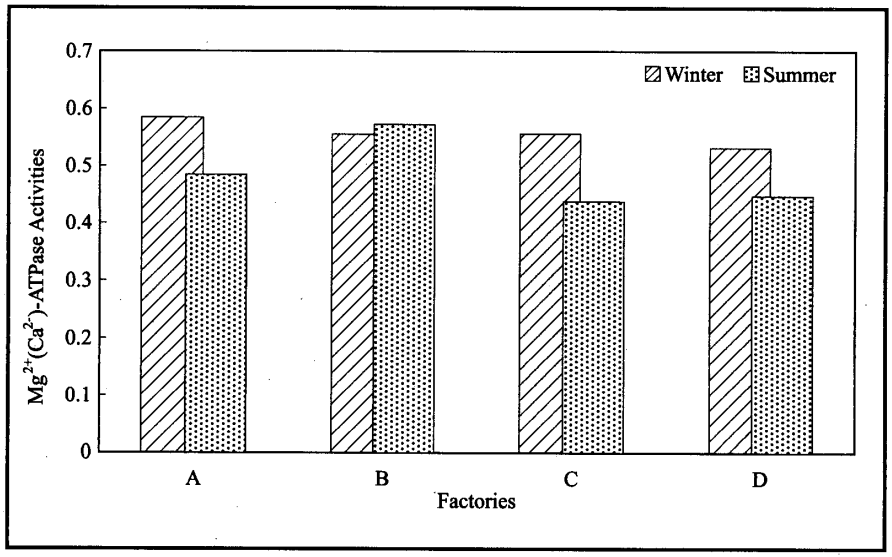

Fig. 6. Changes in Mg^{2+} (Ca^{2+})-ATPase activities of washed meat in the winter and summer season.

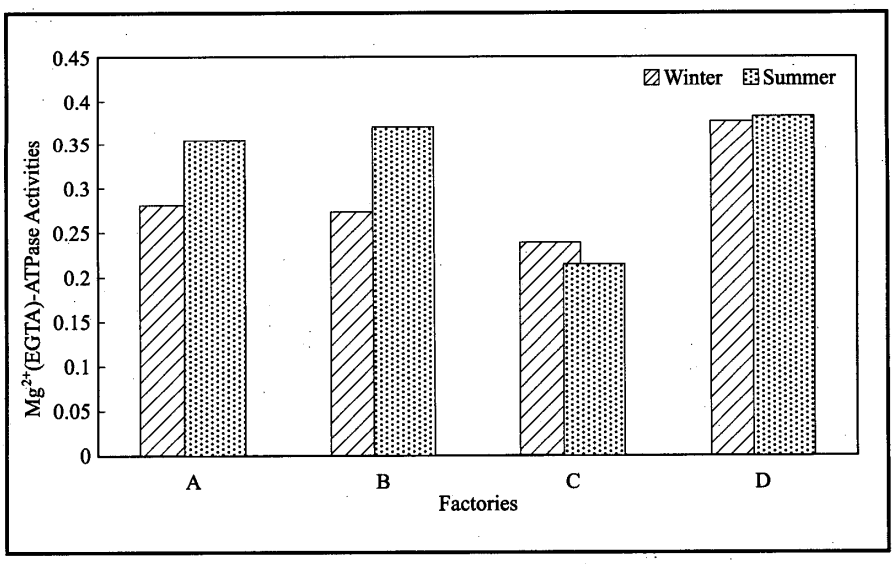

Fig. 7. Changes in Mg^{2+} (EGTA)-ATPase activities of washed meat in the winter and summer season.

tion, but also SH_a on the LMM, was not oxidized during the washing process in the surimi-based products manufacturing of Kochi prefecture.

Conclusion

It was clearly observed that the oxidation of fish meat through the formation of disulfide bonds did not occur strongly during washing process in the surimi based products manufacturings of Kochi prefecture. Therefore, it was suggested that the oxidation of fish meat during washing process is a little and do not affect the gel forming ability of the surimi-based products.

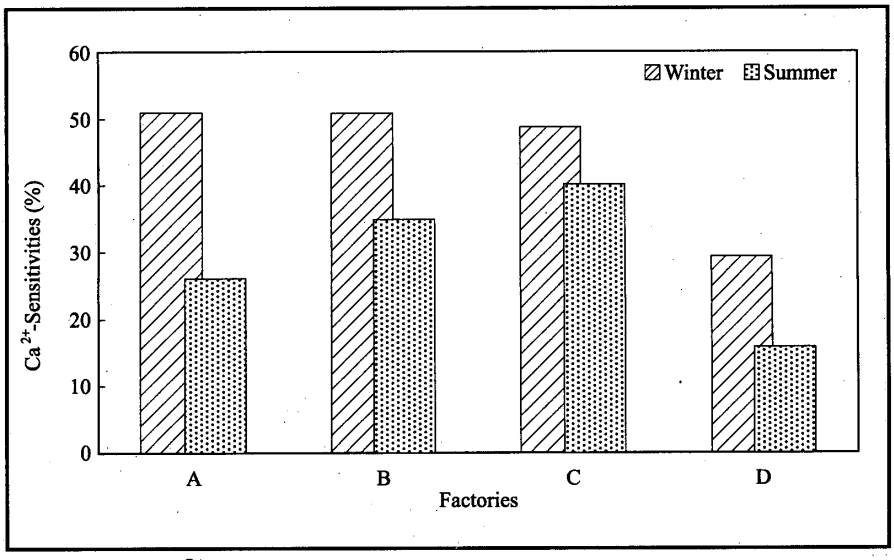

Fig. 8. Changes in Ca^{2+} -sensitivities of washed meat in the winter and summer season.

Acknowledgement

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