Effect of KBrO₃ on Gel-forming Properties of Walleye Pollack Surimi through Setting with or without Transglutaminase Inhibitor

K. Banlue, K. Morioka and Y. Itoh
Laboratory of Aquatic Product Utilization, Faculty of Agriculture, Kochi University, Nankoku, Kochi 783-8502, Japan

Abstract: In order to clarify the effect of oxidation itself on the gel formation of salted-surimi through setting, the gel-forming properties were examined with or without transglutaminase (TGase) inhibitor. The gels were prepared from walleye pollack salted-surimi mixed with KBrO₃ through setting at 30°C (suwari gel) for 2 h prior to heating at 80°C for 20 min (kamaboko gel) in the presence or absence of TGase inhibitor. The gel strength of kamaboko gel increased through setting but KBrO₃ almost did not promote the gel formation of kamaboko gels through setting compared with control. KBrO₃ increased the breaking strength and decreased elongation so that the gel became harder. Even in the presence of TGase inhibitor, where the increase in gel strength during setting was suppressed, KBrO₃ showed the promotion of gel formation, although the gel strength is lower than the gel without TGase inhibitor. Almost, similar behaviors of protein polymerization by disulfide bonds and the oxidation of sulphydryl groups to those in the absence of TGase inhibitor were observed. These results suggest that KBrO₃ enhances the gel forming ability of walleye pollack surimi through the disulfide bonding of myosin heavy chain by oxidation during the setting. In conclusion, the oxidation of salted surimi sol during setting at 30°C can contribute to the gel formation, but its contribution seems not to be cooperative with that of TGase.

Key words: Gel-forming ability, setting, oxidants, sulphydryl groups, disulfide bond, pollack, surimi

INTRODUCTION

The gel physical property of fish surimi products like kamaboko is one of the important qualities. It is well accepted that setting or suwari plays a major role in strengthening surimi gels because of the polymerization of Myosin Heavy Chain (MHC) through ε-(γ-glutamyl)lysine cross-linking by TGase during setting, resulting in the enhancement of surimi gel quality (Benjakul et al., 2003; Gilleland et al., 1997; Kimura et al., 1991; Kumazawa et al., 1995). So, setting procedure is now very popular in surimi product processing.

Additionally, disulfide bond that is another predominant covalent bond contributive to the interaction of protein molecules constitutes a portion of the further development of the gel network during heating at high temperature (Lanier, 2000; Montero and Gómez-Quillén, 1996). The intermolecular disulfide bonding is a result of the oxidation of sulphydryl groups in the presence of oxidants or metal ions (Itoh et al., 1979; Kishi et al., 1995). The addition of KBrO₃ has been shown to improve the strength of gels from fish proteins which otherwise had poor gelling ability (Okada and Nakayama, 1961). The mechanism of this improvement was generally accepted to be via oxidation of sulphydryl compounds to disulfide bonds (Lee et al., 1997) and inactivation of proteinase during heat-setting (Facheco-Aguilar and Crawford, 1994). Therefore, the gel formation of fish protein can be considered to be enhanced by the MHC polymerization by the oxidation of protein sulphydryl groups to disulfide bonds caused by the effect of oxidants as well as by the cross-linking resulted from TGase during setting.

However, the effect of oxidation of surimi protein during setting on the gel formation has not been extensively studied. It is not sure whether oxidants may contribute to the promotion of gel formation synergistically or competitively with TGase, since TGase is known as a SH enzyme.

Therefore, the objective of this study was to investigate the effect of KBrO₃ on gel forming properties of walleye pollack surimi during setting at 30°C with or without a transglutaminase inhibitor.

MATERIALS AND METHODS

Chemical reagents: Potassium bromate (KBrO₃) was obtained from Wako Pure Chemical Industries Co. Ltd, (Osaka, Japan). Ethylene glycol-O,O'-bis(2-aminoethyl)-
Gel preparation: Frozen walleye pollack (Theragra chalcogramma) surimi (SS grade, Maruha- Nichiro Co. Ltd. Japan) was used as a raw material. The surimi measured 76.6 and 14.2% in moisture and protein content, respectively. The surimi that was thawed by leaving at 5°C overnight was chopped for 1 min using a chopper (MK-K-48 Matsushita, Japan). The chopped surimi was mixed with the mixture of chilled water (to adjust 80% moisture content), NaCl (3% against 80% moisture surimi) and KBrO₃ (0, 2 and 4 μmol g⁻¹) with or without 10 mM of EGTA for further 3 min.

The resulting pastes were stuffed into stainless steel cylinder cassettes (3.1 cm diameter and 3.0 cm height) and wrapped by polyvinylidine chloride film. These pastes were incubated at 30°C for 0, 30, 60 and 120 min (suwari gel) prior to heating at 80°C for 20 min (kamaboko gel) and subsequently cooled immediately in ice water for 5 min. The resulting gels were kept overnight at 5°C prior to the analysis of the gel properties.

Gel strength measurement: After keeping gels at room temperature for 2 h, the gel strength (g cm⁻²) was estimated by multiplying the breaking strength (g cm⁻²) and the elongation (ΔL/L₀, ΔL breaking length; L₀ sample length) that were measured by stretching test using a rheometer (Model CR-200D; Sun Scientific Co. Ltd, Tokyo, Japan) according to the method of Shimizu et al. (1981). For each gel, 6 determinations were performed and the mean value and standard deviation were calculated.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE): SDS-PAGE was carried out according to the method of Weber and Osborn (1965), using 3% polyacrylamide gel in a vertical disc-gel system (8.0 cm length, 5 mm diameter).

0.1 g of both surimi and kamaboko gel were homogenized using a Teflon homogenizer at 1,200 rpm for 5 min with 4.5 mL of 0.05 M phosphate buffer (pH 6.8) containing 8 M urea, 2% SDS and 0.036 mM N-ethylmaleimide. The obtained homogenate was boiled for 2 min. After boiling, this dissolved solution was cooled in ice water then, kept overnight before preparing unreduced and reduced samples. In order to prepare the reduced sample, 1 mL of the dissolved solution was mixed with 1 mL of the reagent containing 0.05% bromophenol blue in 50% glycerol, 0.4% SDS, 0.5 M phosphate buffer (pH 6.8) and 30% of 2-mercaptoethanol. The unreduced sample was also prepared in the similar manner using the reagent without 2-mercaptoethanol. Ten microliter of the samples was applied to each disc-gel. The protein was stained by Coomassie Brilliant Blue R 250.

Measurement of protein sulfhydryl groups (PSH): The content of protein sulfhydryl groups in surimi sols and kamaboko gels was determined using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) based on Ellman method (Ellman, 1959). The 0.5 g of surimi sol and kamaboko gel were homogenized by using a Teflon homogenizer at 1,200 rpm for 5 min with 25 mL of 0.1 M phosphate buffer (pH 7.0) containing 8 M urea, 2% SDS, 10 mM ethylene diamine tetraacetate acid (EDTA). Four milliliter of the homogenate was mixed with 0.4 mL of 0.1% DTNB dissolved in 0.1 M phosphate buffer (pH 7.0) containing 8 M urea, 2% SDS and 10 mM EDTA. This reaction mixture was incubated at 40°C for 15 min prior to the absorbance measurement at the wavelength of 412 nm using a Hitachi U-1000 spectrophotometer (Hitachi, Tokyo, Japan). The protein sulfhydryl content was finally calculated using a molar extinction of 13,612 M⁻¹ cm⁻¹ for 2-nitro-5-triobenzoic acid at this wavelength.

Statistical analysis: Analysis of Variance (ANOVA) was performed and the mean values were compared based on Duncan’s multiple range tests (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

First in order to examine the effect of oxidation during setting on the gel formation, the walleye pollack salted surimi was mixed with KBrO₃ (0, 2 and 4 μmol g⁻¹) and set at 30°C for 0, 30, 60 and 120 min (suwari gel) prior to heating at 80°C for 20 min (kamaboko gel). Gel properties of these gels were shown in Fig. 1a and b.

In the case of suwari gel at the concentration of 2 and 4 μmol g⁻¹ KBrO₃, the breaking strength of gels increased more than that of the control gel during setting (p<0.05). The elongation also increased dramatically during the first 30 min more than that of the control gel and subsequently decreased to lower values than that of the control with the extension of setting time (p<0.05). The gel strength that is evaluated by the multiplication of breaking strength and elongation increased noticeably during setting for 2 h and the increase was higher than the control gel (without KBrO₃) (p<0.05). These results indicate that KBrO₃ enhanced the gel formation of walleye pollack surimi during setting at 30°C.

In the case of kamaboko gel at direct heating at 80°C (without setting), the addition of 2 and 4 μmol g⁻¹ KBrO₃ increased the gel strength by 391.1 and 171.5 g cm⁻² respectively, comparing to the control gel.
Fig. 1: Effect of KBrO₃ on the properties of (a) suwari gel and (b) kamaboko gel of walleye pollack surimi with or without EGTA. Suwari gel was prepared by setting at 30°C for 0, 30, 60 and 120 min and kamaboko gel was prepared by heating at 80°C for 20 min after setting at 30°C for 0, 30, 60 and 120 min.

Through setting, the breaking strength of kamaboko gel at both two concentrations of KBrO₃ increased higher than the control gel (p<0.05). However, the elongation of the gel treated with KBrO₃ was lower than that of the control gel, depending on the concentration but regardless of setting time (p>0.05). Then the gel strength of kamaboko gel including KBrO₃ increased through setting. However, KBrO₃ almost did not promote the gel formation of kamaboko gels through setting comparing with the control gel without KBrO₃ except the gel treated with 2 µmol g⁻¹ of KBrO₃ at 60 min setting, where the gel strength was higher than the control gel at the same setting time (p<0.05). These results indicate that KBrO₃ can enhance gel forming ability of walleye pollack surimi with the appropriate concentration and setting time.

The enhancement of gel formation of walleye pollack surimi during setting is known to relate with the cross-linking between MHC by TGase. Then in order to elucidate the contribution of KBrO₃ itself to the enhancement of gel formation during setting, gels were prepared in the presence of 10 mM EGTA to inhibit TGase activity. The gel properties illustrated in Fig. 1 shows that EGTA suppressed the gel formation of suwari gel and kamaboko gel without KBrO₃ with the extension of setting time. In the case of suwari gel, the control gel without KBrO₃ showed little increase in breaking strength, elongation and gel strength, though KBrO₃ added-suwari gel showed the increasing behavior of gel properties even in the presence of EGTA similar to the gel without EGTA.

On the other hand, in the case of kamaboko gel, the values of gel properties of gels without KBrO₃ did not increase or slightly decreased in the presence of EGTA through setting (p>0.05). In the presence of KBrO₃ and EGTA, the breaking strength and the gel strength
increased through setting (p<0.05), though the elongation did not increase. The breaking strength of the gel with both reagents was lower than that of gel without EGTA and the same as the control gel without both reagents. The elongation was lower than the gels without EGTA and slightly higher than the gel with both reagents. The gel strength was lower than the gels without EGTA but higher than the gel with both reagents. Thus, KBrO₃ showed the independent effect on gel formation enhancement through setting. Nevertheless, gel strengthening by the effect of KBrO₃ was weaker than either of the gel treated with KBrO₃ (without EGTA) or the control (without both reagents) (p<0.05).

In order to examine the behavior of proteins in walleye pollack surimi through setting in the presence of KBrO₃ with or without EGTA, the suwari gels and kamaboko gels showed in Fig. 1 were applied to SDS-PAGE analysis. The SDS-PAGE patterns were shown in Fig. 2.

SDS-PAGE patterns of suwari gel without KBrO₃ showed that MHC band intensities decreased with the extension of setting time and the higher molecular weight substances (MHC dimer) than MHC appeared above MHC band in both reduced samples and unreduced samples (Fig. 2a). Furthermore, at the same time, the smaller substances than MHC appeared below MHC. The former results indicate that MHC undergoes polymerization via the formation of non-disulfide covalent bonding, which is known as cross-linking catalyzed by the endogenous TGase (Kimura et al., 1991; Kumanawa et al., 1995). The latter result suggests that the degradation of MHC occurred by protease
(Hosain et al., 2001). Kamaboko gel without KBrO₃ also showed the similar patterns to the suwari gels.

In the case of KBrO₃-added suwari gels, the formation of MHC dimer was observed in the unreduced samples of gels without setting. This dimer was disappeared in the reduced samples. After setting, the disappearance of MHC was observed in the unreduced samples. MHC and MHC dimer appeared in the reduced samples of suwari gels. The MHC dimer was a substance that was formed by non-disulfide bond during setting. These results show that MHC and MHC dimer were polymerized by disulfide bonding during setting. In the case of kamaboko gels including KBrO₃, not only the MHC but also actin were almost polymerized by disulfide bonding regardless of concentration of KBrO₃ and setting time (Fig. 3b, c). These results indicate that KBrO₃ polymerized MHC thorugh disulfide bonding during setting and heating process and polymerized actin mainly during heating process.

In the case of EGTA-added gels, as shown in Fig. 3a, MHC dimer was not observed in both unreduced samples and reduced samples of suwari gels without KBrO₃ as well as in the reduced samples of kamaboko gels without KBrO₃. That is MHC intensity was almost constant during setting. However, MHC dimer and polymer on the top position of disc gels were observed in the unreduced samples of kamaboko gels. These results mean that MHC was not polymerized by non-disulfide bonding during setting but polymerized by disulfide bonding during heating at 80°C.

In the unreduced samples of suwari gel treated with KBrO₃ (4 μmol g⁻¹), MHC and MHC dimer were disappeared during setting and MHC was subsequently recovered in the reduced samples as shown in Fig. 3b. These results mean that most MHC was polymerized to high molecule substances by disulfide bonds upon setting at 30°C.

SDS-PAGE patterns of kamaboko gels with EGTA showed that MHC and MHC dimer as well as actin were completely polymerized by disulfide bonding regardless of setting time as can be seen by comparing the unreduced samples with the reduced samples. These results indicate that KBrO₃ oxidized both MHC and actin to the high molecular substances by disulfide bonding during heating process.

In order to confirm the formation of disulfide bonds in surimi gels and kamaboko gels in the presence of KBrO₃, FSH content was determined and shown in
In the case of kamaboko gels (Fig. 5), PSH content decreased further upon heating in the presence of KBrO₃ of 2 and 4 μmol g⁻¹ by 7.8 to 8.1 and 10.2 to 10.3 μmol g⁻¹, respectively. However, the control gel without KBrO₃ did not show the clear decrease in PSH content upon heating (p<0.05). These results revealed that KBrO₃ oxidized PSH of walleye pollack surimi to disulfide bonds depending on the concentration and setting time. Furthermore, the oxidation of PSH strongly occurred upon heating at 80°C depending on the concentration but regardless of the setting time. Opsvedt et al. (2005) reported that the oxidative effects of KBrO₃ markedly increase corresponding to the rising of temperature. Rungrerdkriangkrai et al. (1999) reported that at high temperature, proteins were unfolded and the intrasulfhydryl groups were exposed to the surface of protein molecules, which coincides with the progress of formation of polymer by disulfide bonds during heating process. Fujita and Itoh (1984) reported that free amino acid cysteine is oxidized to cystine and cysteic acid in the presence of KBrO₃ at 80°C.

In the presence of 10 mM of EGTA, PSH content in suwari gels (Fig. 4) and kamaboko gels (Fig. 5) decreased with extended setting time and exhibited the same behavior as that of gel without EGTA, though the decrease in PSH content was slightly lower than that of gel without EGTA. These results show that the oxidation of PSH occurred under the inhibitory condition of TGase similarly to the case without the inhibitor. However, EGTA might suppress the oxidation of proteins owing to the suppressing of unfolding of proteins by chelating calcium ion that induces the unfolding of proteins (Yongsawatdigul and Sinsuwan, 2007).

In the case of suwari gel, gel formation in the absence of KBrO₃ was suppressed by EGTA (Fig. 1). This result indicates that EGTA suppressed the formation of MHC dimer through non-disulfide cross-linking by TGase, because MHC dimer was not observed in the reduced samples of EGTA-added gels (Fig. 4). The suppression of gel formation by EGTA is due to the inhibition of TGase activity by chelating calcium ions that is essential to the enzyme (Hossain et al., 2001; Wan et al., 1994). On the other hand, the gel formation in the presence of KBrO₃ during setting was not suppressed with EGTA, though the formation of MHC dimer by non-disulfide bonding was suppressed in the presence of EGTA. In spite of the presence of EGTA, similar extent of oxidation of PSH occurred in both gels. These results suggest that the gel formation during setting in the absence of KBrO₃ is promoted by TGase, an endogenous TGase is known to catalyze the polymerization of MHC via the formation of non-disulfide covalent cross-links during setting (Kimura et al., 1991; Kumazawa et al., 1995). But that in
the presence of KBrO₃ is promoted mainly by the intermolecular oxidation of PSH. However, the formation of MHC dimer by non-disulfide bonding during setting in the presence of KBrO₃ (Fig. 2b, c) suggests that TGase in surimi worked some extent even in the presence of KBrO₃.

In kamaboko gel, proceeding of gel formation through setting in the presence of KBrO₃ was suppressed by EGTA some extent regardless of setting time. This difference can be considered to be the contribution of TGase through cross-linking. In addition, the proceeding of gel formation seems to be related with the extent of oxidation of PSH during mixing and setting, not with the extent of the oxidation upon heating. In other words, the oxidation during mixing and setting might be more contributable to the gel formation than the oxidation upon heating at 80°C. The temperature and the processing stage where the disulfide bonds are formed in surimi might have never been discussed.

In the case of gel formation of control kamaboko gel without KBrO₃, the decrease in gel strength through setting was observed in the presence of EGTA. This decrease may be due to the degradation of MHC by protease, because the production of substances between MHC and actin on SDS-PAGE patterns was observed (Fig. 2). The proteolysis observed in this research may be not by Ca-dependent protease, because EGTA chelates only calcium ion. This degradation was slightly suppressed in the presence of KBrO₃, as can be seen in the reduced samples of suwari gels and kamaboko gels in Fig. 2 and 3. This result suggests that the cysteine protease was inhibited by KBrO₃. Walleye pollack surimi is known to include cysteine protease and serine protease (Hu et al., 2008). The inhibition of degradation might contribute to increase gel strength a little, because the decrease in gel strength of gel without KBrO₃ by degradation was not so large.

Now we are under confirming the effect of KBrO₃ on TGase activity in surimi. Furthermore, we are examining which portion of sulfhydryl groups in MHC is oxidized to form MHC dimer or polymer in order to make clear the difference of the oxidation mechanism from the oxidation in the fish meat without salt.

CONCLUSIONS

In conclusion, it was found that the oxidation of salted surimi sol occurred not only upon heating at 80°C but also during setting at 30°C and mixing with salt in the presence of KBrO₃, and that the oxidation during setting prior to heating can contribute to the gel formation through the polymerization of MHC by intermolecular disulfide bonding. However, the contribution of oxidation in surimi seems not to be cooperative with that of TGase.

REFERENCES


