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Effect of pH-Shifting on Setting and Disintegrating Behaviors of Walleye Pollack Surimi

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Abstract: In order to study the effect of pH-shifting of meat on setting (suwari) at 30°C and disintegrating (modori) of fish meat at 60°C, the walleye pollack surimi whose pH was lowered to pH 6.0 and was readjusted to original pH were examined for different preheating time. At 30°C, acidified sample did not increase in gel strength with the prolongation of preheating time. RENEUTRALIZED sample (pH 7.2) increased in gel strength with the increase in preheating time, but the gel strength was weaker than that of the original sample. At 60°C, gel strength decreased with the lapse of preheating time irrespective of pH value. Acidified sample was much weaker compared with the original sample and reneutralized sample was only a little stronger than the acidified sample. These results seem that the suwari was inhibited and modori was promoted, once the surimi was acidified. Then, the effect of pH-shifting to the activity of transglutaminase (TGase) and proteases in surimi, which are known to be related to the suwari and modori, respectively, were examined. Both of transglutaminase and protease activities in reneutralized surimi were the same level as that of the original surimi. These results confirmed that pH-shifting do not denature transglutaminase or protease, irreversibly. Therefore, the decrease in gel strength by pH-shifting might be due to the denaturation of myosin itself which is known as the major protein in gel forming ability.

Key words: Walleye pollack surimi, suwari and modori, acid denaturation, TGase activity, protease activity

INTRODUCTION

It was reported that gel strength is affected by pH-lowering of surimi. Furthermore, it was also observed that the setting (suwari) and disintegrating (modori) behavior varied among different pH value, though the highest gel strength was observed at 30°C and the lowest at 50-60°C irrespective of pH value. Alvarez *et al.*^[1] reported that different combinations of setting and cooking time and temperature determined the textural characteristics of kamaboko gels. In some combinations, gel strength decreased if setting and cooking was prolonged beyond a certain point. For some species extended incubation at certain temperature (generally below 40°C) can enhance the gelation of surimi, whereas for other species extended incubation around 60°C may weaken the surimi gel^[2]. Some researchers reported that extent of cross-linking of MHC in ground meat is notably dependent on the pH for setting^[3].

Several studies have demonstrated that setting effect is correlated with cross-linking reaction of protein, which is mediated by endogenous TGase and results in more

elastic and rigid gels after cooking at high temperatures^[4-6]. TGase in walleye pollack muscle and surimi demonstrate that the endogenous TGase is essentially involved in the setting procedure of surimi gel production and plays a major role in strengthening surimi gels by modifying thermal myosin gelation through ϵ -(γ -glutamyl) lysine cross-linking^[5,7-9].

Various types of proteases are found among fish species, which are optimally active from acid to alkaline conditions^[10]. About 144 proteolytic enzymes of animal tissues have been classified^[11]. These can be divided into two main families: exopeptidases (proteinases), which are restricted to terminal peptide linkages and endopeptidases, which are not restricted. In surimi, the endopeptidases have the more serious effect, because internal cleavage reduces the size of protein polymer and thus its gel-forming ability decreases dramatically. Siebert^[12] pointed out that fish muscle typically exhibits protein autolysis (cleavage by endogenous proteolytic enzymes, "proteinases") at a rate 10 times that of mammalian muscle.

However, information are lacking about the effect of preheating time on suwari and modori behavior of walleye pollack at different pH. Therefore, the investigation was done to examine the effect of pH-shifting on suwari and modori for various preheating times.

In the previous experiments it was found that during setting (30°C) the gel strength was suppressed by pH-shifting treatment which might be due to the denaturation of myofibrillar protein and/or the suppression of endogenous TGase activity of surimi. So, the investigation was also carried out to confirm whether pH-shifting affect the activity of endogenous TGase of surimi for different incubation period.

As the results of the previous experiments showed a decreasing trend in the gel forming ability with the decrease in pH value of surimi, from that trend it was supposed, proteases might be involved with the increase in degraded substances and thus the gel forming ability declined. Then the attempts were taken to confirm whether pH-shifting has any influence in the changes of individual or total amounts of free and bound amino acids during gelation that might cause the decrease in gel strength at 60°C.

MATERIALS AND METHODS

Materials: Walleye pollack frozen surimi (SS1) grade, Maruha Co.Ltd was used as a raw material.

Chemical analysis: Moisture content of surimi was determined by air drying of a given sample in an infrared moisture determination balance (FD-600-2, Kett Electric Laboratory, Japan). Protein content was estimated by multiplying the nitrogen factor of 6.25 to the nitrogen amount determined by micro-Kjeldhal method^[13].

Gel preparation: Walleye pollack surimi was left for thawing at 4°C for about 12 h in the cold room. Then surimi was acidified by using 1N lactic acid from pH 7.2 (original) to 6.0 and was kept for overnight. The next day half of surimi was reneutralized to its original pH 7.2 and was adjusted to 80% in the moisture content. The other half of surimi was adjusted to 80% in moisture content without reneutralizing. Then these surimi were ground with 3% salt for 20 min. The resulting pastes were stuffed into stainless steel cylinder cases (3.1×3.0 cm) and wrapped with polyvinylidene chloride film and preheated at 30 and 60°C for 1, 2 and 3 h prior to heating at 80°C for 20 min. The control sample was heated only at 80°C for 20 min. The produced gels were then cooled in ice-water and stored at 4°C until the gel properties were measured.

Measurement of kamaboko gel properties: Before conducting the stretching test, the gels in stainless cases were kept at a room temperature for 20-30 min. Gel

strength (g cm^{-2}) was assessed by multiplying the breaking strength (g cm^{-2}) and the elongation which were measured by using a Rheometer (Sun Scientific Co., Ltd., Model CR-200D) according to the method of Shimizu *et al.*^[14]. For each sample, 10 determinations were performed and mean values were calculated.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE):

The gels or pastes were homogenized with urea-SDS buffer (8 M urea - 2% SDS - 50 mM phosphate buffer, pH 6.8, containing 0.09% N-ethylmaleimide) and boiled for 2 min and immediately cooled. Unreduced samples were prepared by mixing this solution with the same amount of 0.05% bromphenol blue (BPB) - 50% glycerol - 0.4% SDS - 0.05 M phosphate buffer. Reduced samples were prepared by mixing the homogenate with the same amount of 0.05% BPB - 50% glycerol - 0.4% SDS - 0.05 M phosphate buffer - 20% 2-mercaptoethanol. SDS-PAGE was carried out in 3% polyacrylamide gel according to the method of Weber and Osborn^[15].

Measurement of apparent transglutaminase (TGase) activity in surimi:

The activity of TGase in surimi was measured according to the method of Kanoh *et al.*^[16] with some modifications. The pH of samples was lowered first and then again readjusted to its original pH (7.2). The pH lowered and readjusted samples were then ground with 3% NaCl and 2 mL of monodansylcadaverine (MDC) solution (16.8 mg MDC, 6 mg CaCl_2 and 6 mg dithiothreitol in 4 mL of deionized water) for 10 min. The samples were then heated at 30°C for 0 and 120 min and cooled in ice water, immediately. In the next step homogenation of the samples was done with 19 volumes of phosphate buffer (pH 6.8) containing 8 M urea-2% SDS. Boiling was done at 100°C for 2 min and cooled. Then the samples were subjected to 3% polyacrylamide gel for SDS-PAGE analysis and detected by UV radiation. The emission intensity of the samples was measured by fluorescence spectrophotometer with the excitation of 350 and emission 480 nm.

Measurement of true TGase activity of crude enzyme solution at 30°C:

True TGase activity of the pH-shifted samples was measured according to the method of Tsukamasa *et al.*^[17] with some modifications. The pH of surimi was shifted (accept control sample) using either lactic acid or NaOH and kept overnight at 4°C. Then the samples (5 g) were homogenized for 2 min at 5000 rpm with 5 vol. of 60 mM Tris-HCl buffer (pH 7.5) containing 5 mM EDTA, 10 mM NaCl and 3 mM DTT. The obtained homogenate (crude enzyme soln., 400 μL) was applied to 2 mL of 60 mM Tris-HCl buffer (pH 7.5) containing 2.4 mg casein (N,N-Dimethylated, Bovine), 600 μM MDC, 24 mM 2-mercaptoethanol and 12 mM CaCl_2 . The samples were then incubated at 30°C for 0, 5, 10, 20, 30, 60 and

120 min, mixed with 4 mL of 10% TCA to terminate the reaction and centrifuged for 10 min at 2200×g. After centrifugation the precipitate was washed 4 times with ethanol : ether (1:1) and air-dried. Dried precipitate was solubilized in 4 mL of 50 mM Tris-HCl buffer (pH 8.0) containing 8 M urea-2% SDS and adjusted to 10 mL in volume, which was used as sample solution. Each sample solution was applied to 10% polyacrylamide gel for SDS-PAGE analysis and detected by UV radiation.

Sample preparation for free amino acid analysis: To analyze the free amino acids, first of all the pH of surimi was shifted by lactic acid or NaOH and kept at 4°C for overnight. Then pH-shifted samples (5 g) were homogenized for 10 min at 10,000×g with 5% TCA. Homogenate was filtrated and supernatant was stored at refrigerator until analysis.

Sample preparation for bound amino acid analysis: The samples (2 mL) stored at refrigerator were hydrolyzed against 2 mL of conc. HCl at 110°C for 24 h in DRI-BLOCK BD-3H. Samples were cooled for about 30 min and transferred to round bottom flask for evaporation at EYELA Rotary Vacuum Evaporator. To solubilize the samples, 2 mL 0.01 N HCl was added and solubilized samples were stored in refrigerator until analysis. Analysis of amino acid samples were done by a standard amino acid analyzer (HITACHI Amino Acid Analyzer; L 8500).

RESULTS AND DISCUSSION

Preheating time-gelation curves of kamaboko gels at 30 and 60°C: Changes in breaking strength, elongation and gel strength of SS1 grade walleye pollack surimi at pH 6.0,

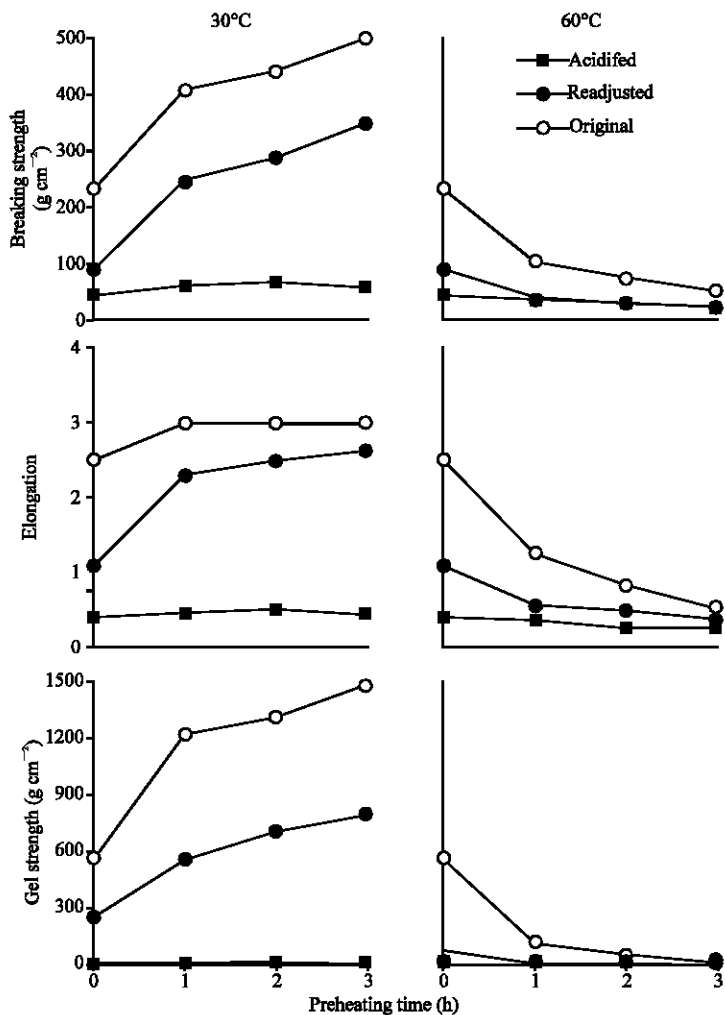


Fig. 1: Effect of preheating time on breaking strength, elongation and gel strength of pH-shifted SS1 grade pollack surimi at 30 and 60°C

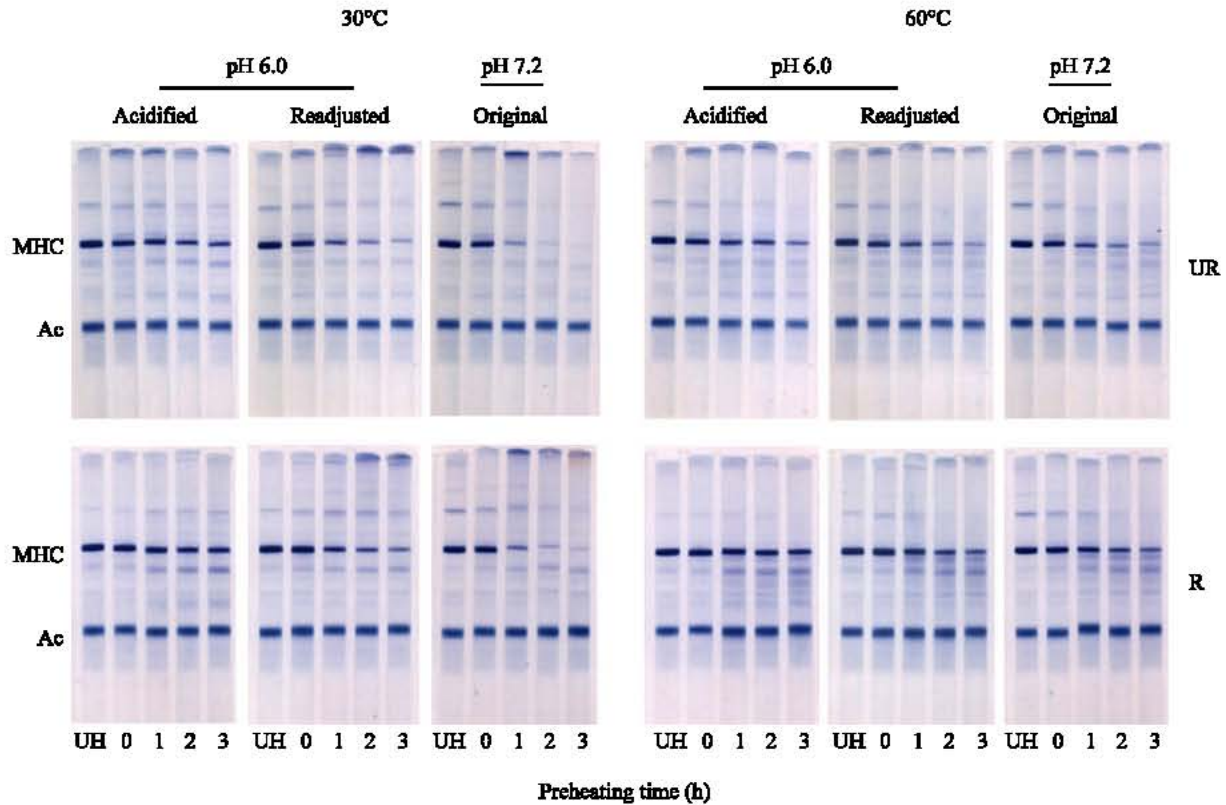


Fig. 2: SDS-PAGE pattern of walleye pollack surimi gels preheated for different preheating time at 30 and 60°C, prior to heating at 80°C 20 min at pH 6.0, 7.2 (readjusted from 6.0) and 7.2

7.2 (readjusted from 6.0) and at pH 7.2 during preheating at 30 and 60°C for various preheating time are shown in Fig. 1. The breaking strength, elongation and gel strength increased with the duration of preheating time at 30°C, irrespective of pH value. At pH 7.2 the gel strength increased with the increase in preheating time at 30°C. The highest gel strength was obtained about 1500 g cm⁻² after 3 h preincubation. Alvarez and Tejada^[8] elucidated that the increase in gel strength at ≤35°C is time dependent and in walleye pollack has been associated with cross-linking which occurs among MHC.

When the pH value lowered to 6.0, the gel strength was greatly affected showing the value about 30 g cm⁻². Even 3 h preheating could not contribute to improve the gel strength. It is likely that addition of lactic acid to surimi paste caused the denaturation of myofibrillar proteins or suppressed the activity of endogenous TGase enzymes. Ishizaki *et al.*^[9] claimed that the interfacial denaturation of actomyosin was very sensitive to pH value. The readjustment of pH from 6.0 to 7.2 resulted in an increase in the gel strength with the prolongation of preheating time showing the maximum value 800 g cm⁻² after 3h preheating. This result suggests that myofibrillar

protein might be denatured due to lowering the pH from 7.2 to 6.0. However, denaturation occurring at 30°C might not be so severe that the gel strength could be improved significantly by readjusting the pH from 6.0 to 7.2.

At 60°C, the breaking strength, elongation and gel strength decreased with the prolonged duration of preheating time at 60°C regardless of pH value (Fig. 1). At pH 7.2, gel strength gradually decreased with the progress in preheating time at 60°C. The lowest gel strength was obtained about 30 g cm⁻² after 3 h preheating. Many researchers reported a gel weakening at around 55-60°C in various fish species^[20-22]. When the pH value was lowered to 6.0, the gel strength was severely affected. Even the readjustment of pH from 6.0 to 7.2 could not improve the gel strength. These results indicated that decrease in gel strength at 60°C might be the outcome of the combined effect of myofibrillar denaturation and proteolysis by some heat stable proteases at pH 6.0.

SDS-PAGE pattern of the pH-shifted kamaboko gels preheated for different time at 30 and 60°C: SDS-PAGE pattern of the samples showed a decrease in myosin heavy chain (MHC) at pH 7.2 with the prolongation of

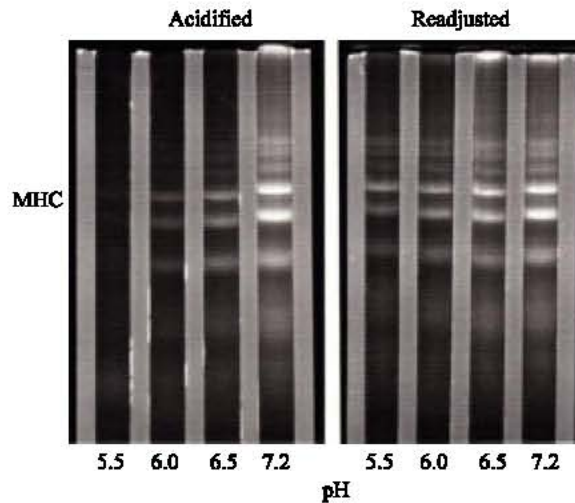


Fig. 3: Incorporation of MDC into proteins of surimi at different pH. MHC, myosin heavy chain

preheating time at 30°C (Fig. 2). This decrease in MHC could be largely attributed to the formation of MHC polymers, particularly since levels of smaller-molecular-weight components (evidence of possible proteolysis) did not appreciably increase. With the increase in preheating time after 60 min or above, probably a greater percentage of MHC polymers formed was too large to enter into the 3% acrylamide gel or was not solubilized prior to electrophoresis^[5]. Numerous researchers^[24-26] have attributed such MHC polymerization during setting to an enzymatic reaction, catalyzed by TGase, which produces cross-linking in myosin through covalent bonds. There is a positive correlation between cross-linking of MHC and gel forming ability^[27].

At pH 6.0 (Fig. 2) degradation of MHC (reduced samples) increase somewhat with the progress of preheating time and the cross-linking of MHC (unreduced samples) did not occur strongly. However, cross-linking of MHC could be improved and degradation could be reduced some extent by readjusting the pH from 6.0 to 7.2. High molecular weight cross-links were clearly observed on the top of the acrylamide gels at pH 7.2 (readjusted from 6.0 to 7.2) during preheating above 1 h (Fig. 2). These components were considered resulting from cross-linking of MHC^[28-30].

The SDS-PAGE patterns of the samples at pH 7.2 preheated at 60°C showed a decrease in MHC (unreduced samples) and an increase in degraded substances (MHC-A) (reduced samples) with the prolongation of preheating time (Fig. 2). Degradation of MHC occurred strongly at pH 6.0 (Fig. 2, reduced samples) in compare to the gels at pH 7.2. However, degradation could be reduced some extent by readjusting the pH from 6.0 to 7.2 (reduced samples). Lanier^[31] reported that most fishes

possess heat-stable proteolytic (protein-degrading) enzymes (proteases), which disintegrate the protein network and attack the muscle proteins most actively during the cooking of the surimi seafood, when the temperature is between 50 and 70°C. Some of these heat-stable proteases are most active at higher pH, whereas others are most active at pH 5.5. However, all are still quite active over the usual pH range of most surimi and minced fish, which is between 6.0 and 7.5.

From the results it was suggested that suppression of gel strength by pH-shifting treatment might be due to the denaturation of myofibrillar protein and/or the suppression of endogenous TGase activity. On the other hand, during gel softening (60°C), decrease in gel strength might be occurred due to the denaturation of myofibrillar protein and/or the action of proteolysis by proteases. Therefore, to confirm whether pH-shifting effect the activity of TGase and protease enzyme of surimi, further experiments were carried out. Results and discussion on the confirmation experiments are sited below.

Effect of pH-shifting to the apparent activity of TGase in surimi: Incorporation of MDC into proteins (mainly myosin) of surimi at different pH is shown in Fig. 3. This Fig. 3 shows that a decreasing trend was observed in the fluorescence intensity with the decrease in pH value of the samples. While the alkaline treatment was done i.e., the pH value of the sample was readjusted from 5.5, 6.0 and 6.5 to 7.2, the fluorescence intensity increased gradually. But original fluorescence intensity observed at 7.2, could not be regained. This result suggests that the pH-lowering affect the TGase activity in surimi.

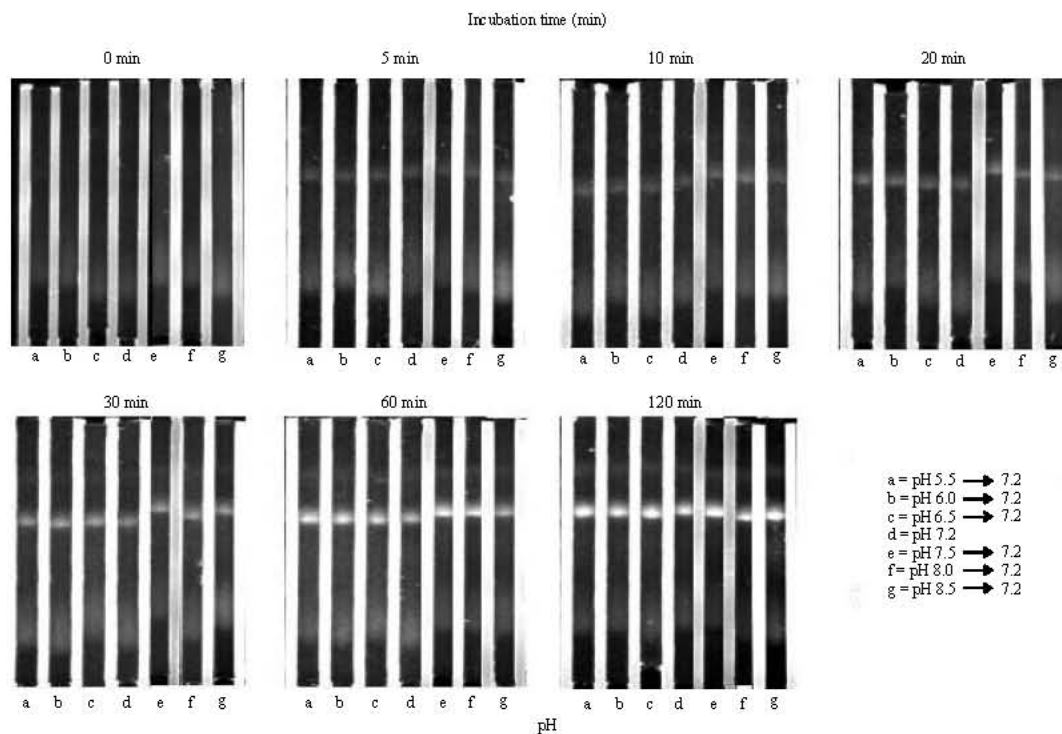


Fig. 4: Incorporation of MDC into casein with crude TGase enzyme samples for different incubation time at various pH (sample amount, 100 μ L)

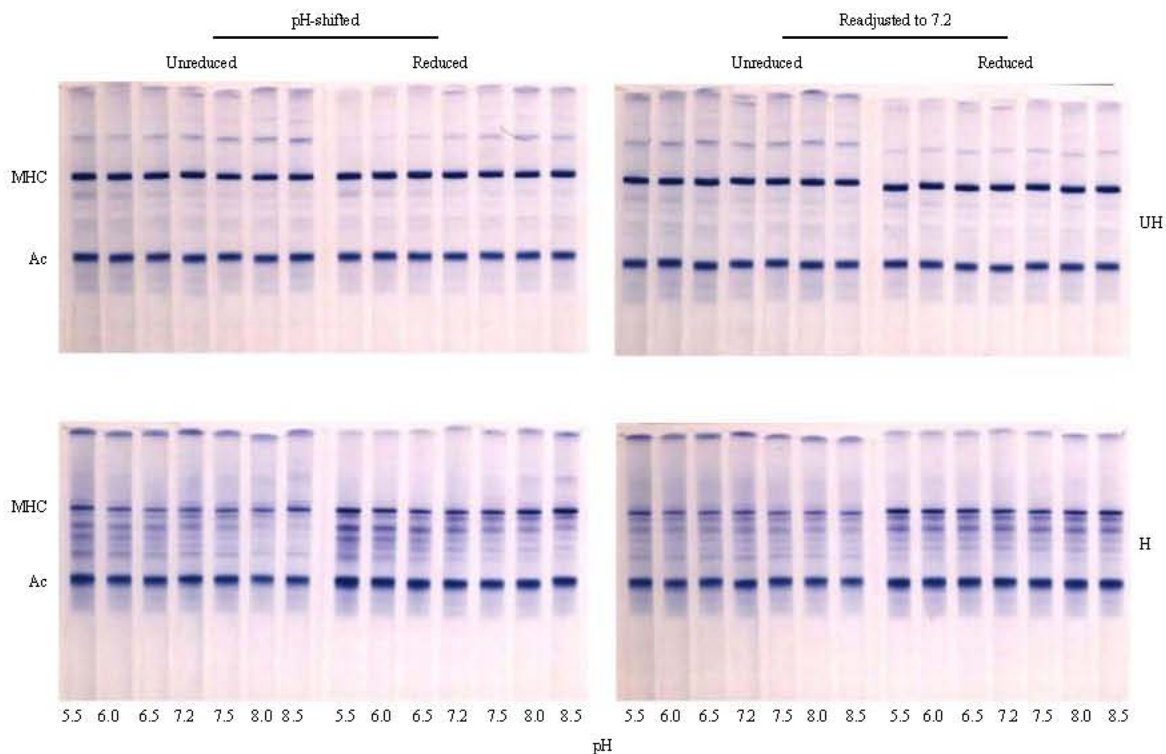


Fig. 5: SDS-PAGE pattern of walleye pollack surimi gels preheated at 60°C for 2 h prior to heating at 80°C for 20 min at different pH and at pH 7.2 (readjusted from different pH). Meaning of the symbols are same as described for Fig. 2

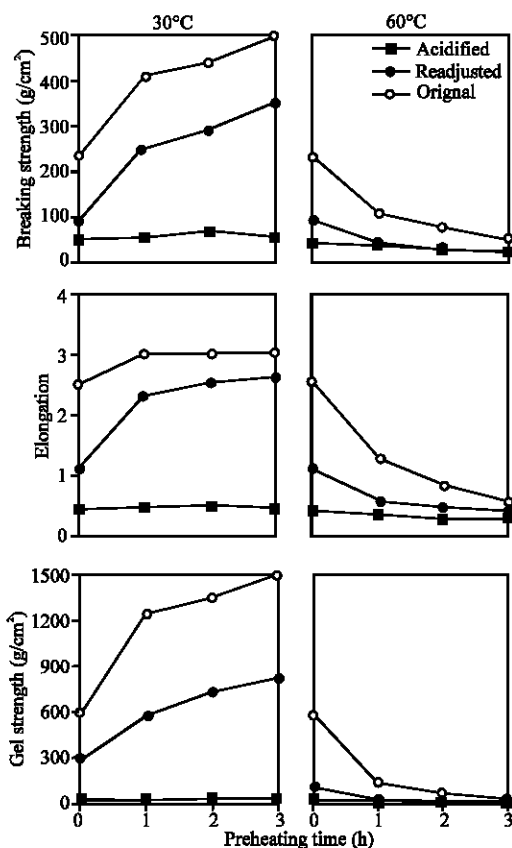


Fig. 6: Total amounts of free amino acids (FAA) and peptides in walleye pollack surimi at different pH

Effect of pH-shifting to the true activity of TGase in surimi: Figure 4 shows the incorporation of MDC into casein with curde TGase enzyme samples for different incubation time at various pH. Figure 4 shows that with the increase in incubation period incorporation of MDC into casein increased irrespective of samples.

On the other hand no differences could be observed in incorporation of MDC into casein with crude TGase enzyme sample at pH 7.2 or pH-readjusted samples from different pH, indicating that pH-shifting could not affect the true activity of TGase enzyme in surimi significantly, i.e., TGase was not denatured by pH-shifting treatment but myosin was denatured.

SDS-PAGE pattern of surimi gels preheated at 60°C for 2 h prior to heating at 80°C for 20 min at different pH: SDS-PAGE patterns of unheated samples at different pH (Fig. 5) showed that the intensity of protein band in both unreduced and reduced condition was almost constant irrespective of pH value, indicating that neither polymerization nor the degradation of MHC occurred in unheated samples by shifting the pH value.

On the other hand SDS-PAGE pattern of heated samples showed that when pH-shifting treatment was done with the decrease in pH value the amount of degraded substance increased and the highest degradation occurred at pH 5.5. Afterward with the increase in pH value a decreasing trend in degradation was observed. Among the readjusted pH samples no remarkable difference was observed in the polymer content of unreduced samples or degraded substances in reduced samples. This result suggests that proteases might not be affected irreversibly by the pH-shifting of surimi.

Total amounts of free amino acids (FAA) and bound amino acids (peptides) in surimi at different pH: Figure 6 shows the total amounts of free amino acids and bound amino acids both for unheated and heated samples at different pH or at pH 7.2 (readjusted from different pH). This figure clearly indicates that pH-shifting most likely could not affect the total amounts of either free amino acids or bound amino acids of unheated samples. In heated samples, the increase in the total amounts of bound amino acids observed due to the acidic treatment and highest value was observed at pH 5.5 which was demoted after the readjustment of sample pH to its original, indicating that proteases were not activated irreversibly by pH-shifting.

From the present results it was confirmed that TGase was not inactivated and proteases were not activated irreversibly, by pH-shifting. Therefore, the decrease in gel strength by pH-shifting at 30 and 60°C might be due to the denaturation of myosin itself which is known as the major protein in gel forming ability.

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