INTRODUCTION

Kamaboko gel is formed from fish meat paste or surimi paste by heating after the suwari and modori stages. Each stage involves a characteristic process with a combined physicochemical and enzymatic reaction. The pH of the salted paste is one of the most important factors in producing a strong elastic kamaboko gel. The optimal pH of the salted paste for strong gelation is pH 6.5–7.0 for flyingfish muscle paste, as reported in early studies by Shimizu et al.1

Miyake and Tanaka have reported that the optimal pH for pelagic fishes such as mackerel, tuna and yellowtail is 6.2–6.7 and is 7.0–7.5 for white meat fishes such as walleye pollack, flatfish, Japanese sea bass and grunt.2 Furthermore, horse mackerel muscle paste forms a strong gel at pH 7.0, but Lan et al. have reported that catfish muscle paste yields its highest gel strength at pH 6.0 compared to other pH values.3

The optimal pH for all mammalian, chicken, turkey muscle pastes is 6.0.3 The heat-induced gelation of purified myosin from rabbit muscle is optimally developed at pH 6.0,4 and this pH dependence by myosin gelation is derived from that of myosin rod, but not S1. S1 gelation is independent over a wide range of pH from 5.0 to 8.0.5

Kim et al. have compared the gelling properties of walleye pollack surimi and beef myofibrils as a function of pH and found that the maximum gel strength was obtained at pH 7.0 for surimi and pH 6.0 for beef myofibrils.6 Mammals, avian, freshwater fishes and some pelagic fishes are classified

ABSTRACT: The effect of pH on thermal gelation and transglutaminase (TGase; EC2.3.2.13)-induced suwari (setting) of surimi and actomyosin pastes was investigated. A strong and elastic gel was produced from walleye pollack surimi paste at pH 7.0 in the presence of Ca\(^{2+}\) using a two-step heating method. In contrast, walleye pollack actomyosin paste formed a weak gel under the same conditions as a result of the low concentration of endogenous TGase. In the presence of EGTA [ethyleneglycol bis(2-aminoethylether) tetraacetic acid], weak gels were formed at pH values of 7.0 and 6.0. Non-proteolytic modori (gel weakening) occurred extensively in the course of actomyosin gelation, but not in surimi gelation. Maximum TGase-induced myosin heavy chain cross-linking was observed at a slightly higher pH of 7.5 than at the optimal pH of endogenous TGase activity; the difference being derived from different substrates. Gelation of carp actomyosin paste at pH values of 5.5, 6.0, 6.5 and 7.0 was monitored by measuring storage modulus (G\(\prime\)) and loss modulus (G\(\prime\)\(^{-}\)). A weak gel was formed at all pH values, but a slightly rigid and less elastic gel was obtained at lower pH values. The addition of microbial TGase (MTGase) formed strong elastic gels at pH 7.0 and 6.5. MTGase cross-linked myosin heavy chains even at pH 5.5, but contributed neither to suwari response nor strong gel formation. Overall, results suggest that the optimal pH for the gelation of surimi paste from easy-setting fish species is a compromise between the pH-optima of TGase activity and of preferable actomyosin conformation for myosin cross-linking.

KEY WORDS: actomyosin, gelation, kamaboko, modori, pH, setting, surimi, suwari, trans-glutaminase.
as non-suwari species whose meat pastes show optimum gelation below pH 7 (pH 6.0–6.7), as mentioned earlier. But the optimum pH for white meat fishes belonging to easy suwari species is approximately 7.0. These species differences in pH dependence are supposed to be partially linked to the various ways heat-induced gelation can be affected by suwari.

Suwari is a unique property of muscle paste or surimi paste that is derived from some white meat fish species and has been attributed to the TGase-catalyzed cross-linking of myosin heavy chains. Therefore, the optimal suwari pH must be compatible with that of TGase activity. However, the various effects of pH on the thermal gelation of meat paste and on TGase-induced gelation have not been assessed well. Therefore, the aim of the present study is to compare the effects of pH on the thermal gelation of pastes in the presence and absence of TGase activity or in an enzyme inhibitory system.

MATERIALS AND METHODS

Materials

Frozen walleye pollack Theragra chalcogramma surimi (SA grade), which contained 4% sucrose, 4% sorbitol and 0.2% polyphosphates, was produced on board the ship Ocean Phoenix (Pacific Seafoods Inc., Seattle, USA) and imported by Nippon Suisan (Tokyo, Japan). The surimi was stored at –40°C for 4–10 months until required for use. Protein concentration was approximately 180 mg/g wet weight. Live carp Cyprinus carpio and walleye pollack were purchased from a local store. Microbial TGase was supplied by Ajinomoto Co. Inc. (Tokyo, Japan); EGTA was obtained from Dojindo Laboratories (Kumamoto, Japan); and monodansylcadaverine was purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals, which were of analytical grade, were from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of actomyosin and surimi pastes

Carp and walleye pollack were filleted and skinned. The dorsal muscle was minced and washed three times in four volumes of 0.1 M NaCl–20 mM Tris-HCl (pH 7.0). The washed muscle was homogenized in five volumes of the same buffer. After centrifugation, the precipitated myofibrils were washed four times with the same buffer and passed through a layer of gauze to remove connective tissue. Myofibrils were ground with NaCl to prepare actomyosin paste containing 90 mg protein/g of paste and 0.6 M NaCl at the final concentration. The pH of the actomyosin paste was lowered slowly and adjusted to a fixed pH by the addition of 0.1 M HCl while grinding in an ice bath just before heating. The required amount of 0.1 M HCl was predetermined by titrating spare actomyosin paste with HCl.

The method of Wan et al. was followed to prepare the surimi paste. Frozen surimi was thawed and chopped in a food processor for 4 min with 0.6 M NaCl–20 mM Tris-HCl (pH 7.0) at the final concentration. Protein concentration was adjusted to 90 mg/g with the addition of H2O, and pH was adjusted with the addition of 0.1 M HCl as described previously.

Determination of enzyme activity and optimal pH

Walleye pollack TGase was extracted from muscle and partially purified using DE52 cellulose chromatography and Sephacryl S-300 gel filtration. Transglutaminase activity was measured according to Nozawa et al. using acetylated or succinylated casein and monodansylcadaverine as substrates. Transglutaminase activity in surimi paste was measured using the method of Takeda and Seki. To determine the optimal pH for TGase-induced myosin cross-linking, the relative velocity of the disappearance of myosin heavy chain monomers was measured using surimi paste of various pH values by adding 0.1 M HCl or NaOH.

Gelation and rheological measurement

To measure gel strength, actomyosin and surimi pastes were de-aerated, packed into plastic vessels (3.7 cm diameter, 2 cm height), and then heated using a two-step heating method in which the samples were heated at 25°C for 0 h, 2 h, 4 h, 6 h and 8 h before heating at 90°C for 20 min. Heated samples were chilled immediately in iced water for 1 h, kept at room temperature for 2 h and then removed from the vessels. Breaking strength was measured by using a cylindrical plunger (5 mm diameter) at a penetration speed of 0.5 mm/s in a rheometer (Rheon RF3305; Yamaden, Tokyo, Japan). The force required to rupture the gel was thus determined.

Dynamic rheological changes of actomyosin and surimi pastes during thermal gelation were analyzed using a Rheolograph Sol rheometer (Toyoseiki Seisakusho, Tokyo, Japan). Sample (1.6 mL) was heated linearly from 5°C to 80°C at 2°C/min or
using a two-step heating method consisting of linear heating from 5°C to the suwari temperature (30°C and 40°C for carp actomyosin paste; 25°C for walleye pollack actomyosin and surimi pastes) for 60 min, followed by linear heating up to 80°C at a heating rate of 2°C/min. G’ and G” values were recorded as described elsewhere. Reproducibility of data was confirmed with duplicate runs.

SDS-PAGE

Protein samples were solubilized with 2% sodium dodecylsulfate (SDS), 2% 2-mercaptoethanol, 8 M urea and 50 mM Tris-HCl (pH 8.0). Sodium dodecylsulfate–polyacrylamide gel electrophoresis was performed in 2% acrylamide disk gels containing 6 M urea as described elsewhere.

Protein determination

Protein concentration was determined according to the biuret method using bovine serum albumin (BSA) as a standard. Surimi and muscle pastes were solubilized in 1 M NaOH at room temperature prior to the determination.

RESULTS AND DISCUSSION

Gelation of walleye pollack surimi paste and actomyosin paste at pH 7.0 and 6.0

To investigate the effect of endogenous TGase on the gelation of walleye pollack surimi paste at pH 7.0 and 6.0, the gelling process was followed by changes in dynamic viscoelasticity during heating from 5°C to 80°C at 2°C/min, including suwari at 25°C for 120 min (Fig. 1a). For comparison, the paste’s enzyme activity was inhibited by the addition of EGTA, a calcium chelator, as reported by Wan et al. At pH 7.0 in the presence of Ca2+, the surimi paste showed the highest G’ value even before heating. The G’ value increased sharply after suwari and reached a maximum of 2300 Pa at 80°C, forming a rigid surimi gel. In the presence of EGTA at the same pH, the paste’s G’ value was approximately 60% of that with Ca2+ at the initial stage and decreased gradually during suwari and increased slightly at the final stage. The G’ value at 80°C was only 700 Pa, indicating the formation of surimi protein aggregate but not a gel. The Ca2+ effect on gelation is consistent with the results of Wan et al.

At pH 6.0, the surimi paste showed a low G’ value during suwari and increased to 1000 Pa at 80°C regardless of the whether Ca2+ was present or absent. Results indicated that the rigidity of the gel formed at pH 6.0 was higher than that at pH 7.0 in the presence of EGTA.

The G’ value of the pastes at both pH values changed similarly during heating and decreased to below 100 Pa at 80°C (data not shown), as reported elsewhere.

As commercially produced surimi contains various additives such as sugars and polyphosphates, we carried out the experiments using walleye pollack actomyosin paste to avoid any complexities derived from the unexpected effects of the additives on gelation. Furthermore, endogenous TGase was almost, but not completely, removed from actomyosin during preparation. The G’ value of the actomyosin paste at pH 7.0 in the presence of Ca2+ increased during suwari and decreased sharply at 53°C. At 80°C, the G’ value of the actomyosin gel was only 1100 Pa, which was and is indicated in the figure by numerals 7 and 6 for pH 7.0 and 6.0, respectively. Ca and EG show the pastes in the presence of CaCl2 and EGTA, respectively. MTGase (1.25 unit/g) was added to actomyosin paste at pH 7.0 (7 M) or pH 6.0 (6 M). (----) Temperature.
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Regardless of the suwari period, SDS-PAGE revealed that myosin heavy chains were cross-linked to a great extent at pH 7.0, but not at pH 6.0 (Fig. 3). The actomyosin paste was slightly gelatinized during suwari at pH 7.0 because of the formation of a small amount of cross-linked myosin heavy chains, which was catalyzed by the remaining endogenous TGase in the paste (Figs 2b, 3). The addition of MTGase completely recovered the breaking strength of the actomyosin gel to a higher level, which was comparable to that of surimi at pH 7.0. Furthermore, at pH 6.0, MTGase increased the breaking strength of the actomyosin gel to a higher level than that of surimi in the presence of Ca$^{2+}$. Microbial TGase also cross-linked myosin heavy chains to a great extent at pH 6.0.

Figure 4 shows walleye pollack endogenous TGase activity compared with pH. The enzyme activity's pH dependence was measured in two systems: (i) in the casein–monodansyl cadaverine system with partially purified enzyme; and (ii) for myosin heavy chain cross-linking activity in surimi paste in the presence of 5 mM CaCl$_2$. The former system showed that the optimal pH of the enzyme activity was approximately 7.0. Walleye pollack enzyme activity decreased sharply from pH 7.0 to pH 6.0, in which the activity was only 11% of the maximum. Alternatively, the latter system showed the optimum to be at pH 7.0 for myosin cross-linking and essentially no cross-linking activity at pH 6.0. The discrepancy between the optimal pH values of the two systems may be because of the

![Graph](image-url)

**Fig. 2** Effect of pH, Ca$^{2+}$ and transglutaminase (TGase) on the breaking strength of walleye pollack surimi and actomyosin gels. (a) Surimi and (b) actomyosin gels were formed using a two-step heating method as follows: suwari was performed at 25°C for 0 h, 2 h, 4 h and 6 h, and then heated to 90°C for 20 min. Please refer to Fig. 1 legend for symbols.
different substrates. The rate of myosin cross-linking may be influenced by the specific conformational states of substrate at various pH values as well as enzyme conformation. Microbial TGase has a broad optimal pH ranging 6.0–7.0, as reported by Ando et al. The marked difference in activity levels at pH 6.0 between endogenous TGase and MTGase seemed to be reflected in the different gel strengths of the surimi and actomyosin pastes with MTGase at pH 6.0 as shown in Fig. 2 [compare (a) 6Ca with (b) 6M]. Several researchers have reported that walleye pollack surimi gel with maximal gel strength is formed at pH 7.3.

Overall, the results suggest that TGase-induced suwari is an essential process to producing a strong elastic gel and, therefore, the optimal pH for gelation must almost coincide with the optimal pH of TGase activity and a preferable conformation of the substrate protein involved.

It is well established that non-proteolytic modori is characterized by a temporal decrease in G’ at approximately 50°C unless proteolysis is detected by means of SDS-PAGE Non-proteolytic modori occurred during the gelation of walleye pollack actomyosin (Fig. 1b); however, it was not detected during the process of surimi gelation regardless of the pH and Ca²⁺ concentrations (Fig. 1a). Although the cause of the difference remains unclear, this finding seems to be a clue to better understanding the mechanism of non-proteolytic modori.

**Effect of pH on the gelation of carp actomyosin paste**

As carp actomyosin paste is more stable than that of walleye pollack, actomyosin prior to suwari

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**Fig. 4** Effect of pH on endogenous transglutaminase (TGase) activity. (○) The activity of TGase, which was partially purified from walleye pollack muscle, was assayed in medium containing 0.1 mM monodansyl cadaverine, 1.0 mg/mL succinylated casein and 10 mM CaCl₂ at 25°C and at various pH values. (○) Myosin heavy chain cross-linking activity was measured in the surimi paste containing 90 mg protein/g-paste, 0.6 M NaCl and 5 mM CaCl₂ at 25°C for 1h and at various pH values (mean value ± SD; n = 4).
and heating is expected to keep a native state even at lower pH values for a short period of time for paste preparation. An endogenous TGase activity was not detected in carp actomyosin. Figure 5 shows the changes in the dynamic viscoelastic properties of carp actomyosin paste at various pH values during heating from 5°C to 80°C at a constant heating rate of 2°C/min. A typical G’ profile was obtained at pH 7.0; G’ increased at 30°C, showed two transitions, and increased again at temperatures above 53°C. Although similar profiles were shown at pH 6.5 and 6.0, the temporal drop point was shifted to lower temperatures; that is, 49.4°C and 46°C at pH 6.5 and 6.0, respectively. The profile was quite different at pH 5.5; G’ was lowest below 50°C and increased above 44°C until reaching 80°C at the highest value of 1000 Pa.

The G” value of actomyosin paste at pH 7.0, 6.5 and 6.0 increased gradually until 28°C and then decreased sharply to bottom at approximately 55°C. At pH 5.5, G” was minimum at 52°C and increased markedly between 52°C and 65°C. Carp actomyosin gels became more rigid and less elastic at lower pH values because of a decrease in the electrostatic repulsion among protein molecules and because of thermal denaturation. The water holding capacity of muscle gel decreases in all species, responding similarly with a decrease in pH.3,4

When the suwari procedure at 30°C for 60 min was introduced to carp actomyosin gelation, the changes in the G’ profile and final magnitude were essentially the same as those of linear heating at all pH values except that the maximal value at 80°C was obtained at pH 6.0. During 40°C suwari, G’ was increased markedly by an increase in pH, although the final G’ magnitude was similar for all pH values (Fig. 6). Results in Figs 5 and 6 clearly demonstrate

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Fig. 5  Effect of pH on the thermal gelation of carp actomyosin paste. Actomyosin paste containing 90 mg protein/g-paste, 0.6 M NaCl and 5 mM CaCl₂ was heated at the heating rate of 2°C/min from 5°C to 80°C at the various pH values indicated in the figure. (----) Temperature.

Fig. 6  Effect of suwari on the thermal gelation of carp actomyosin paste at various pH values. The suwari process at 30°C and 40°C for 60 min was introduced to thermal gelation as shown in Fig. 5.
that the suwari procedure was ineffective in increasing gel strength at all pH values.

**Effect of pH on the TGase-induced suwari of carp actomyosin paste**

Microbial TGase (5 unit/g) -added carp actomyosin paste at various pH values was set at 30°C and 40°C for 60 min (Fig. 7). As the pH of the paste increased, G' values increased during suwari and increased again after the temporal drop. At 80°C, the highest G' value was obtained at pH 7.0, followed by, in descending order, pH 6.5, 6.0 and 5.5. A strong elastic gel was formed at pH 7.0 and 6.5 with a G' value of 2500 Pa. Even at pH 6.0, a gel with a G' value of 2000 Pa was formed and the suwari effect was more pronounced at 40°C than at 30°C. Microbial TGase-induced suwari was ineffective at pH 5.5. SDS-PAGE (Fig. 8) revealed that MTGase cross-linked myosin heavy chains to a great extent at pH 7.0. Microbial TGase was still active at pH 5.5 and cross-linked myosin heavy chains but did not contribute to gelation, as shown in Fig. 7, because of a conformational state of actomyosin at a low pH. As reported in other studies, the breaking strength of carp actomyosin gel increased with an increase in the activity level of added MTGase. The
G* value of actomyosin gel with MTGase decreased sharply after suwari and showed a small peak at approximately 65°C at pH 5.5 and 6.0 (Fig. 7). This peak formation suggests the temporal loss of water retention. The high G* and G" values of actomyosin gel at 80°C and at low pH values showed a tendency to form a more rigid and less elastic gel.

The pH adjustment of TGase-induced thermal gelation is an important process for maintaining an optimal state in the enzyme and actomyosin conformations in order to produce a strong elastic gel matrix. It is therefore suggested that the optimal pH of TGase is the chief determining factor for the optimal gelation of easy suwari fish species. Gelation of carp paste, whereby carp tor for the optimal gelation of easy suwari fish, improved gel properties with increased softness. Therefore, the pH for the optimal gelation of fish paste is determined as generally being 6.5–7.0.

REFERENCES