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Effect of setting conditions on mechanical properties of acid-induced Kamaboko gel from squid *Todarodes pacificus* mantle muscle meat

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Abstract Squid *Todarodes pacificus suwari* gels, set at various temperatures and times, and acid-induced kamaboko gel, which was prepared by soaking *suwari* gel in 5% acetic acid for 20 h, were studied to evaluate the mechanical properties that are affected by setting conditions. Unset squid meat paste did not form a gel when soaked in acetic acid. The breaking strength of both *suwari* gel and acid-induced kamaboko gel showed a tendency to increase with setting temperature and time. SDS-PAGE analysis of *suwari* gel and acid-induced kamaboko gel, which were set at various temperatures and times, showed that myosin heavy chain (MHC) was observed at 30°C only for the first hour. The intensity of the MHC band at 30°C gradually decreased with setting time, while the intensity of the polymer band gradually increased with setting time. These results suggest that the protein-protein bonds in *suwari* gel affect the final texture of acid-induced kamaboko gel. Based on the analysis of the mechanical properties, and in consideration of the fact that the purpose of this experiment was to reduce energy usage, the best setting condition was determined to be 40°C for 3 h.

Keywords *Suwari* gel · Acid-induced kamaboko gel · Squid · Setting conditions · Mechanical properties

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Introduction

Japanese common squid *Todarodes pacificus* is one of the most important fishery products in Japan. Approximately 500,000 tons of squid is caught in Japanese waters every year, and the catch is estimated as 4.5% of that generated by the entire Japanese fishery [1]. Approximately 40% of the squid supply is marketed as fresh or frozen for sashimi, sushi, and home-cooking. The remainder is processed into a wide variety of products, including *ika-kamaboko* (squid-based kamaboko), in response to consumer demand [2].

In general, kamaboko is produced via a multi-step process. First, surimi is mixed with salt to induce dissociation of actomyosin [3]. Next, to improve kamaboko gel strength, which is one of the characteristics associated with high-quality surimi products [4], functional additives such as egg white are added [5]. Additionally, the setting process is often optimized by incubation of the surimi at temperatures lower than 40°C in order to enhance gel strength [6]. The resultant gel product is called *suwari* gel [7]. Finally, the *suwari* gel is boiled, steamed, or baked at more than 80°C to produce kamaboko.

In industrial manufacturing, the heating process involves high costs, both in terms of fixed and variable costs, such as those from electrical and other utilities. At the same time, a method that does not require a heating process is available for making kamaboko. This method has been traditionally used for making fish meat gel in the Kyushu region of Japan, generating a product called *sujime-kamaboko* [8]. The principle behind making this product is similar to typical kamaboko; however, in the case of *sujime-kamaboko*, *suwari* gel is soaked in vinegar or an acetic acid solution at a low temperature as the final cooking step instead of heating [9]. Thus, *sujime-kamaboko* can be called acid-induced kamaboko gel.

The setting step is one of the most important steps in producing a surimi-based product for the preparation of *suwari* gel. During setting, it is generally thought that the strength of the gel matrix is mostly derived from intermolecular interactions, although covalent bonds resulting from transglutaminase action have been reported [10]. From investigations into the effect of the initial network formed during setting on the texture of set and cooked gels, it was concluded that there is a range of optimum setting temperatures and times that ensures kamaboko gels have good textural characteristics [10], and these optimum conditions vary according to the fish used [11].

The gel-forming ability of cephalopod muscle is very low due to its high proteolytic ability and low endogenous transglutaminase activity [12]. It has been reported that metalloprotease is the major protease of Japanese common squid mantle muscle [13] and is characterized by optimum pH activity at 7.0 and optimum temperature of 40°C [14]. Usually, sodium chloride is used in kamaboko production for impasting the fish muscle. However, in the case of squid mantle muscle meat, sodium chloride promotes the protease activity and has been reported to cause very poor texture in kamaboko [15]. Therefore, the use of organic salts as solubilizing agents, such as sodium gluconate (a known chelator), is preferred to sodium chloride [15].

Shortage of endogenous transglutaminase results in a gel of poor texture due to an insufficient amount of enzyme to catalyze the formation of a covalent cross-linking network within the gel structure; therefore, the gel does not set during the setting period resulting in texture problems in kamaboko [12]. The use of microbial transglutaminase is another possible means of improving gelation. Moreover, some studies have reported that protease inhibitors and microbial transglutaminase can be advantageously combined in fish species with poor gel-forming ability [16].

In the present study, the first aim was to demonstrate the necessity of using microbial transglutaminase and replacing sodium chloride with sodium gluconate in acid-induced gelation of squid mantle muscle meat. Secondly, the effect of setting conditions on the quality of acid-induced kamaboko gel was investigated in terms of certain mechanical properties and SDS-PAGE patterns of *suwari* gel and acid-induced kamaboko gel from squid meat.

Materials and methods

Samples

Japanese common squid *Todarodes pacificus* caught from the Japanese Sea off the coast of Tsushima, Nagasaki, in July 2009, was purchased from the local market within 12 h of capture, packed in ice, and brought to the laboratory under ice storage conditions on the day following the catch. The average weight of the squid was approximately 200 g. The squid was degutted and skinned at temperatures below 10°C in order to get the mantle muscle meat. The proximate components of raw squid mantle muscle meat were moisture 78.2 ± 0.05%, crude protein 16.5 ± 0.01%, total lipids 1.01 ± 0.14%, crude ash 1.59 ± 0.03%, and crude carbohydrate 2.65%. The mantle muscle meat was then minced with a meat chopper (Meat Chopper M-22A, Nantsune, Osaka, Japan). Sucrose was added to the minced mantle muscle meat and mixed well at a final concentration of 5% (w/w). The product of this step is called surimi. About 100 g of surimi was then packed into a polystyrene bag and kept at −50°C until use.

The effects of sodium gluconate and microbial transglutaminase on *suwari* gel and acid-induced kamaboko gel formation

The method of acid-induced kamaboko gel preparation was modified from Abe et al. [8]. There were four conditions to be studied. For the first condition, frozen surimi was thawed, chopped, and then tempered for 10 min using a grinding machine (Ishikawa-shiki no. 20, Ishikawa Kojo, Tokyo, Japan). A 1% sodium chloride solution was added and homogenized by the grinding machine for 15 min. After that, 1% microbial transglutaminase (Activa® TG-K, Ajinomoto, Tokyo, Japan) and 3% powdered egg white (Nippon Colloid, Tokyo, Japan) were added, and these ingredients were homogenized for 15 min more. During these steps, the temperature of the squid meat was controlled below 10°C. The squid paste was shaped in a stainless steel vessel with a 32 mm inside diameter and 30 mm length. The squid meat paste was put in a water bath (Eyela NTT-2400, Tokyo Rikakikai, Tokyo, Japan) at 40°C for 3 h to set the meat paste. The product of this step is called *suwari* gel. The *suwari* gel at each setting condition was removed from the vessel, soaked in 5% acetic acid, and put in the water bath (Fine Thermo F-002DA, TGK, Tokyo, Japan) at 15°C for 20 h. The final product is called acid-induced kamaboko gel.

For conditions 2–4, the preparation was the same but differed in the type and amount of salt and amount of microbial transglutaminase. In condition 2, sodium chloride was not added. In condition 3, sodium chloride was replaced with sodium gluconate. Condition 4 was similar to condition 3 except that microbial transglutaminase was not added. The appearance of *suwari* gel and acid-induced kamaboko gel obtained under these conditions was studied.

The effects of setting condition of *suwari* gel and acid-induced kamaboko gel

The preparation of *suwari* gel and acid-induced kamaboko gel was the same as described above. Addition of sodium chloride, sodium gluconate, and/or microbial transglutaminase was determined based on the results of the previous step. Setting temperatures were 30, 40, and 50°C and setting times were 0, 0.5, 1, 3, 6, and 12 h.

pH of *suwari* gel and acid-induced kamaboko gel was measured by homogenizing 10 g *suwari* gel or acid-induced kamaboko gel with 90 g water with a homogenizer (Heidolph Diax 600, Heidolph Instruments, Schwabach, Germany) and measuring with a microprocessor pH meter (pH211, Hanna Instrument®, Woonsocket, RI, USA).

Gels were measured for their breaking strength (g) and breaking deformation (cm) with a rheometer (Rheoner RE-3305-I, Yamaden, Tokyo, Japan) equipped with spherical plunger (diameter 5 mm) with a 1 mm/s raising rate of the sample table. Every measurement was repeated five times, and the mean values were presented.

Samples for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were prepared using a method similar to *suwari* gel and acid-induced kamaboko gel preparation step. Instead of shaping in the stainless steel vessel, the squid paste was shaped into a thin film pattern (8.5 × 2.0 × 0.2 cm) to guarantee that the acid penetrated the gel thoroughly. The samples were solubilized with SDS-urea solution containing 2% SDS, 8 M urea, 50 mM Tris-HCl (pH 8.81), and 2% mercaptoethanol by heating at 100°C for 2 min and dialyzed overnight in SDS-urea solution that did not contain mercaptoethanol. SDS-PAGE was conducted as described by Laemmli [17] using 7.5% separating polyacrylamide gels (PAGEL®, Atto, Tokyo, Japan). Gels were stained with Coomassie brilliant blue R250. Images of the scanned gel were analyzed by the ImageJ software (available at http://rsbweb.nih.gov/ij/). The areas under the myosin heavy chain peak of *suwari* gel and acid-induced kamaboko at each setting condition were compared with unset *suwari* gel and acid-induced kamaboko gel and calculated as the percent of relative content of myosin heavy chain.

Results

The effects of sodium gluconate and microbial transglutaminase on *suwari* gel and acid-induced kamaboko gel formation

The appearance of *suwari* gels and acid-induced kamaboko gels is presented in Table 1. *Suwari* gels and acid-induced kamaboko gels with the addition of either sodium chloride (condition 1), microbial transglutaminase (condition 2), or sodium gluconate (condition 4) did not set. In contrast, *suwari* gel with the addition of sodium gluconate and microbial transglutaminase (condition 3) was observed to form a very weak gel. In addition, acid-induced kamaboko gel under condition 3 formed a soft gel.

The effects of setting condition on *suwari* gel and acid-induced kamaboko gel

The pH of *suwari* gel and acid-induced kamaboko gel was 6.54 ± 0.13 and 3.97 ± 0.28, respectively.

After soaking in acetic acid for 72 h, squid meat paste (setting time = 0) was not set and was of very poor quality in terms of both visual appearance and texture. The mechanical properties of squid meat paste both before and after soaking in acid could not be measured; therefore, the results of the unset *suwari* gel and acid-induced kamaboko gel are not shown in the figures below.

As shown in Fig. 1, the breaking strength of *suwari* gel increased with increasing temperature and setting time (30°C, 8.64–31.04 g; 40°C 55.68–84.61 g; 50°C 129.70–188.54 g). Assessment of the breaking deformation of *suwari* gel, as illustrated in Fig. 2, gave different results from breaking strength. At 30 min, the *suwari* gel at

Table 1 Appearance of *suwari* gel and acid-induced kamaboko gel prepared under various conditions

Condition		Gel type	Appearance
1	1% sodium chloride + 1% microbial transglutaminase	*Suwari* gel	Gel was not set
		Acid-induced kamaboko gel	Gel was not set
2	1% microbial transglutaminase	*Suwari* gel	Gel was not set
		Acid-induced kamaboko gel	Gel was not set
3	1% sodium gluconate + 1% microbial transglutaminase	*Suwari* gel	Gel was very soft
		Acid-induced kamaboko gel	Gel was soft
4	1% sodium gluconate	*Suwari* gel	Gel was not set
		Acid-induced kamaboko gel	Gel was not set

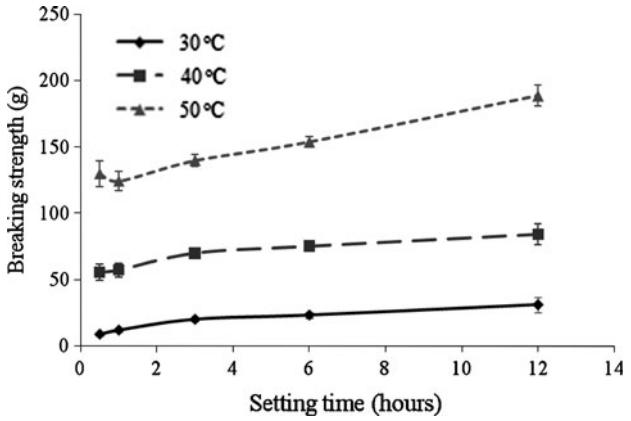

Fig. 1 Effect of setting time on breaking strength of *suwari* gel. Data are shown as mean ± standard deviation. The gel at 0 h is not shown because it could not be measured

40°C showed the highest breaking deformation, while the others showed little or no difference in breaking deformation with increasing time. The acid-induced kamaboko gel set at 30°C had a slimy surface, whereas this was not observed in the acid-induced kamaboko gels set at 40 and 50°C.

The breaking strength of the acid-induced kamaboko gels set at the various temperatures increased with increasing setting time, with the exception of the gel set at 50°C for 12 h, which was slightly less strong than that set at 50°C for 6 h, as shown in Fig. 3 (30°C, 64.96–83.28 g; 40°C, 100.48–132.48 g; 50°C, 155.65–188.54 g). The breaking deformation of acid-induced kamaboko gel is shown in Fig. 4. After soaking in the acetic acid solution, the breaking deformation of the gel set at 30°C was greatest regardless of setting time, whereas the values did not differ among the gels set at 40 and 50°C, regardless of heating time.

SDS-PAGE analysis of *suwari* gel and acid-induced kamaboko gel is illustrated in Fig. 5. In both *suwari* gel and acid-induced kamaboko gel, at a setting temperature of 30°C and a setting time of less than 3 h, MHC bands decreased in intensity with setting time. However, at a setting temperature of 30°C and a setting time greater than 3 h, and at the setting temperatures of 40 and 50°C, MHC bands were not visually detected; however, polymer bands were clearly observed. Moreover, there was a band between myosin heavy chain and paramyosin at 30°C. This band and the paramyosin band also decreased in intensity with a longer setting time, similar to that seen with MHC. Figure 6 illustrates the changes in myosin heavy chain content. It was also shown that myosin heavy chain content was reduced with setting time. The myosin heavy chains of *suwari* gel set at 50°C for longer than 6 h and acid-induced kamaboko gel set at 40°C for longer than 6 h and at 50°C for longer than 3 h were not detected.

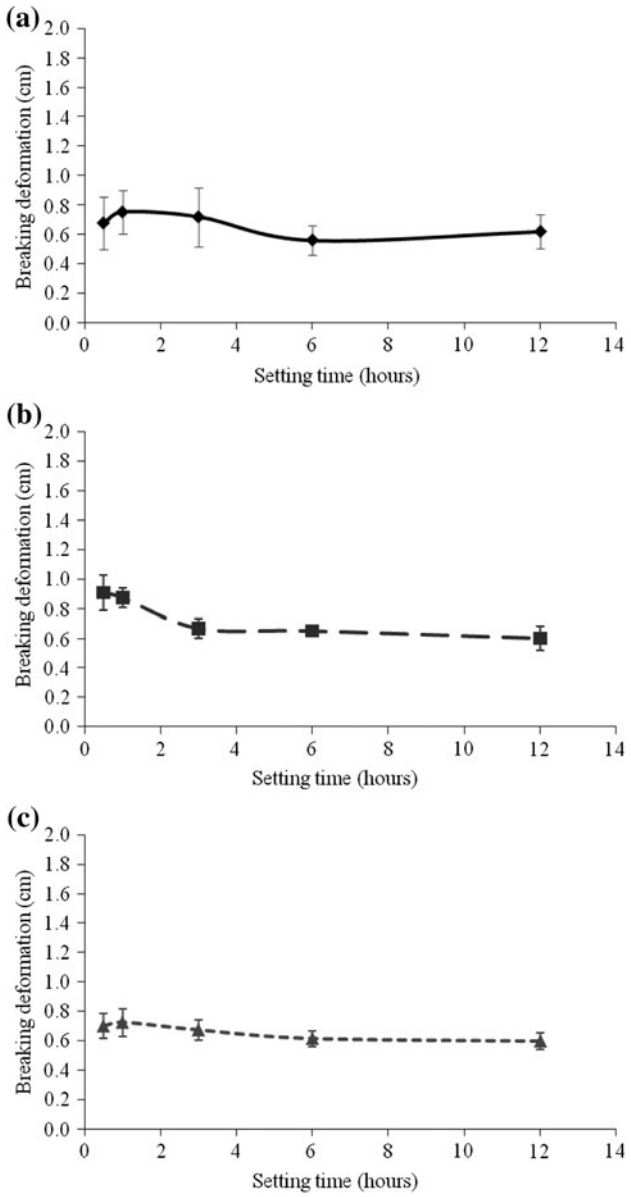

Fig. 2 Effect of setting time on breaking deformation of *suwari* gel. Data are shown as mean ± standard deviation. **a** Setting at 30°C; **b** setting at 40°C; **c** setting at 50°C. The gel at 0 h is not shown because it could not be measured

Discussion

Sodium gluconate and microbial transglutaminase were both necessary for preparing acid-induced kamaboko gel. While sodium chloride promotes protease activity in squid mantle muscle meat, sodium gluconate inhibits this activity via a chelating mechanism [15]. Microbial transglutaminase also improves gelation by catalyzing the formation of extensive covalent crosslinking, chiefly involving myosin heavy chain [12]. Sodium gluconate, as a protease inhibitor, and microbial transglutaminase must be combined in

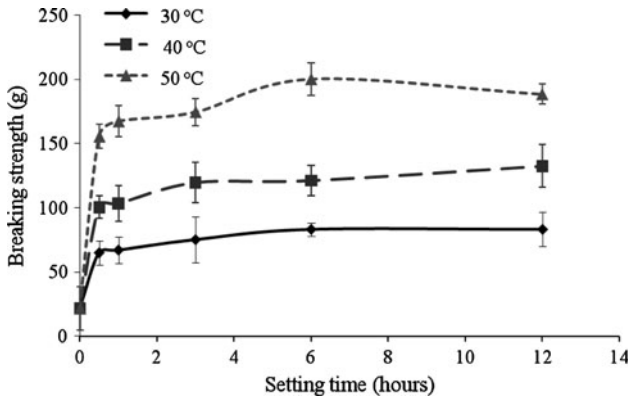

Fig. 3 Effect of setting time on breaking strength of acid-induced kamaboko gel. Data are shown as mean ± standard deviation. The gel at 0 h is not shown because it could not be measured

order to produce *suwari* gel. Moreover, it was clearly observed that if the *suwari* gel did not set, the acid-induced kamaboko gel would not set either. This suggests that the setting step is essential for the production of acid-induced kamaboko gel from squid mantle muscle meat.

During the setting step, myofibrillar proteins establish links with adjacent molecules by covalent bonding, compacting the gel structure [18] via transglutaminase catalysis, and leading to the strengthening of the *suwari* gel. When soaking *suwari* gel in acetic acid, proteins were denatured or unfolded, which occurred subsequent to the formation of the network structure [19]. At 30°C, *suwari* gel was not completely set in the studied time; therefore, the breaking strength of *suwari* gel at 30°C was very low. After soaking in acetic acid solution, the acid-induced kamaboko gel showed approximately 50 g higher breaking strength than *suwari* gel at each setting time; however, the appearance was poor with a slimy surface. This may have been due to the myofibrillar protein in *suwari* gel heated at 30°C not being set completely, thereby unfolding due to the acid effect and subsequently refolding into a nonnative structure due to the effect of the acid anion (acetate ion) [20]. Acid-induced kamaboko gel from *suwari* gel set at 40°C did not have a slimy surface and also showed approximately 50 g higher breaking strength than *suwari* gel at each setting time. On the other hand, the changes in breaking strength at 50°C were obviously smaller than the rest. It was possible that the muscle protein was denatured and aggregated during setting at 50°C; therefore, the formation of the bonds induced by acetic acid may be hard to achieve. Moreover, excessively high temperature and a long setting time caused a reduction in gel breaking strength of acid-induced kamaboko gel. This decrease may be due to destruction of the gel matrix network, associated with the *modori* phenomenon [10] caused by cysteine protease [21]. Taking into consideration the breaking strength result only, the best setting condition would be 50°C for 6 h.

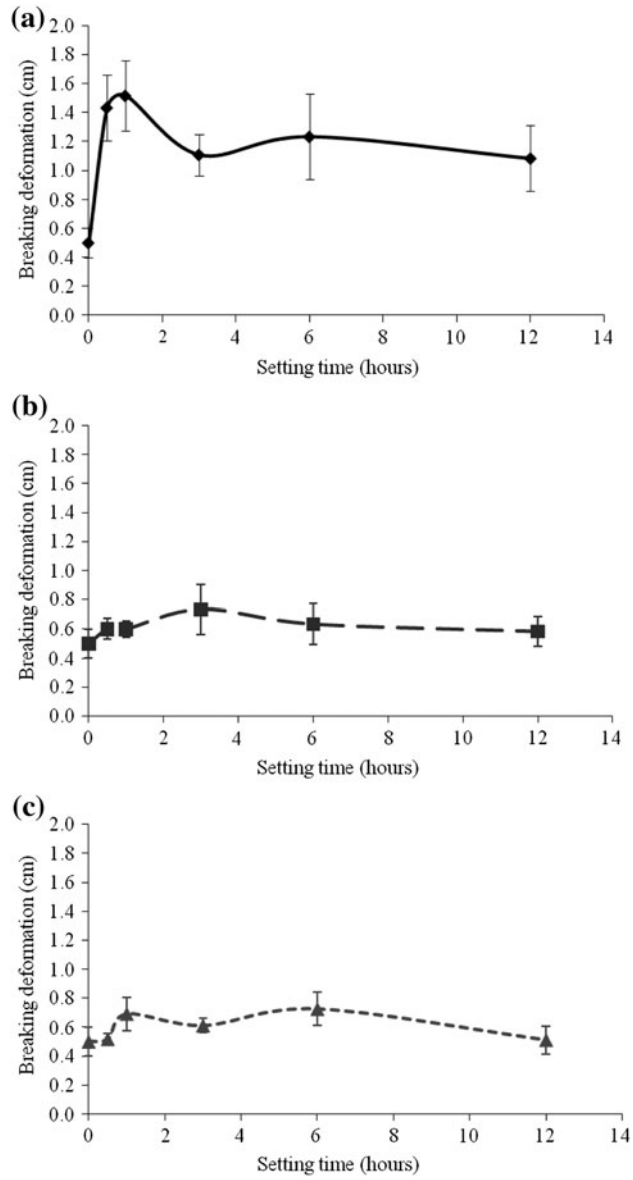

Fig. 4 Effect of setting time on breaking deformation of acid-induced kamaboko gel. Data are shown as mean ± standard deviation. **a** Setting at 30°C; **b** setting at 40°C; **c** setting at 50°C. The gel at 0 h is not shown because it could not be measured

Increasing the setting temperature and time caused a decrease in the breaking deformation of *suwari* gel. This may be because the gel matrix tightened and formed a closer network [22], leading to a reduction in breaking deformation. However, at 30°C the gel was not completely set; therefore, the breaking deformation of *suwari* gel at that temperature was low, similar to its breaking strength. During the soaking of *suwari* gel in acetic acid solution, the acid penetrated the gel structure, forming a more compact protein network and thereby conferring more brittle

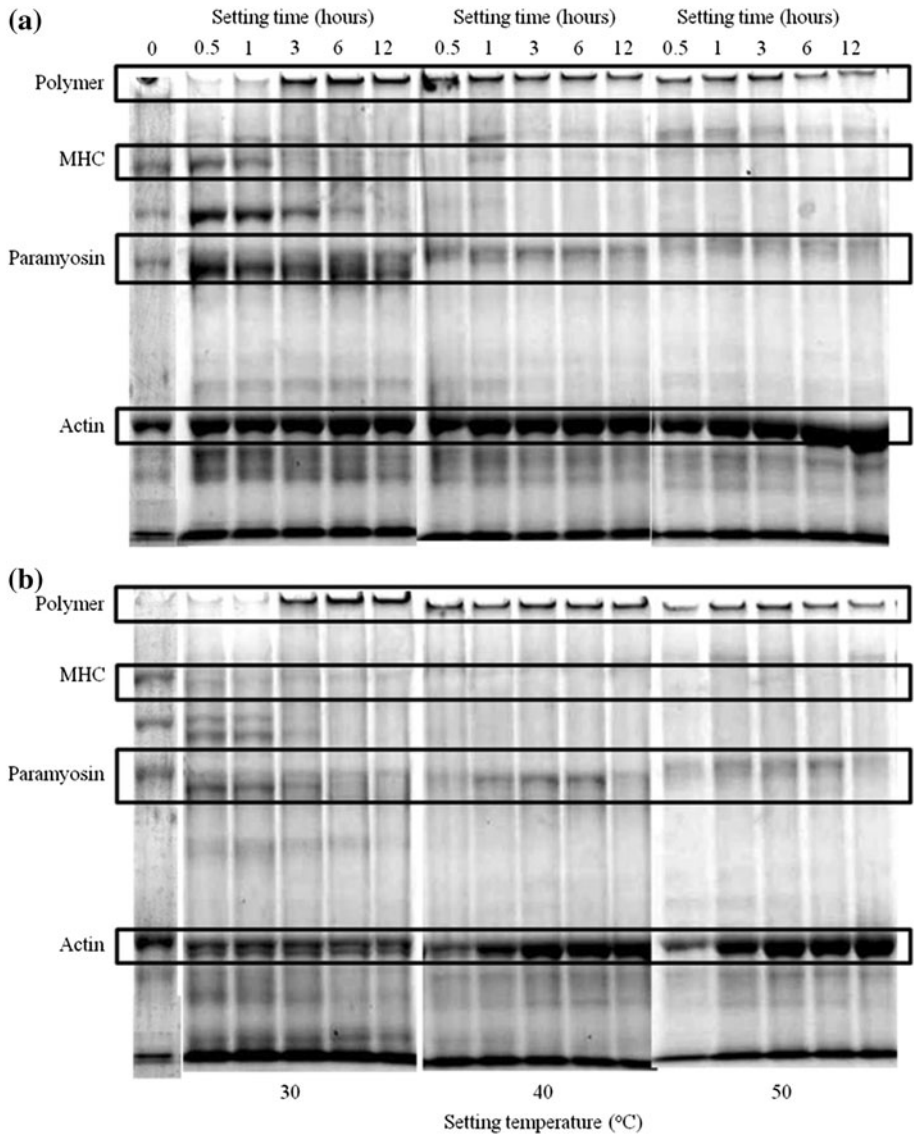

Fig. 5 SDS-PAGE patterns of myofibrillar protein in *suwari* gel and acid-induced kamaboko gel at various setting times and setting temperatures. **a** *Suwari* gel; **b** acid-induced kamaboko gel

characteristics [10]. Taking into consideration the breaking deformation result only, the best setting condition would be at 30°C for 1 h.

Taking into consideration both the breaking strength and breaking deformation results, the best setting condition for acid-induced kamaboko gel from squid mantle muscle meat was 40°C for 3 h. Acid-induced kamaboko gel at 30°C gave high breaking deformation; however, as previously mentioned, the appearance was very poor and could not be used in production. Although the acid-induced kamaboko gel at 50°C gave a higher breaking strength than acid-induced kamaboko gel at 40°C, and although their breaking deformation values were similar, the high temperature was in conflict with the purpose of the study, which was to reduce energy costs. Therefore, the gel at 50°C was not selected. A setting time of 3 h was chosen because the resultant acid-induced kamaboko gel had the highest breaking deformation, while the breaking strength did not differ from gels set for longer times.

The SDS pattern suggested that crosslinking of the myosin heavy chain occurred continuously during setting. It was observed that a decrease in MHC band intensity was inversely related to the intensity of the polymer band. A high degree of protein aggregation is mediated by transglutaminase activity [12]. Microbial transglutaminase activated the crosslinking of MHC [23], which is one of the factors involved in the gelation of kamaboko [24]. Because the optimum temperature of microbial transglutaminase is 50°C [25], protein aggregation occurring in *suwari* gel set at 30°C was slower than that of gels set at 40 and 50°C.

There are several reports that revealed that the absence of MHC bands and the presence of polymer bands were

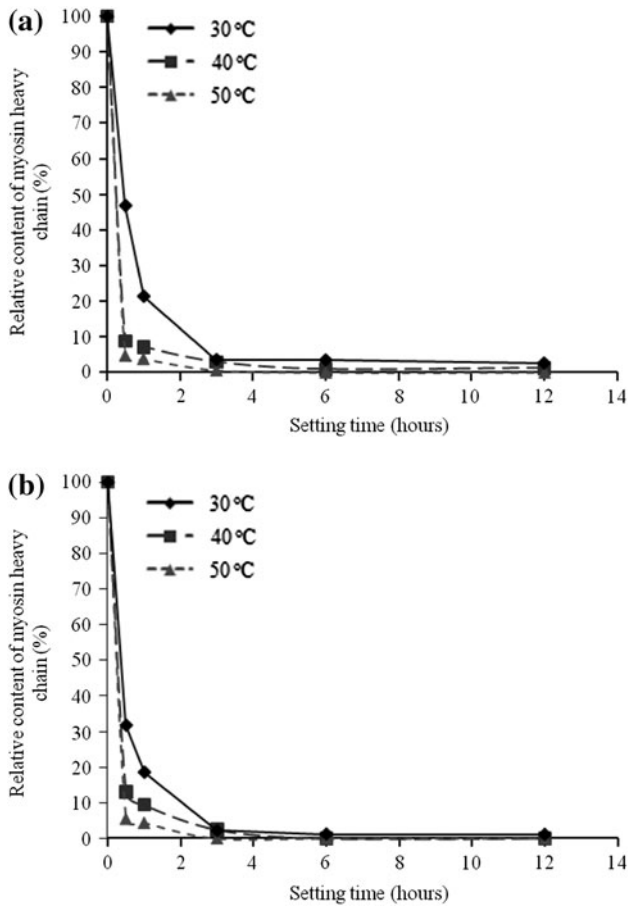

Fig. 6 Changes in the content of myosin heavy chain in *suwari* gel and acid-induced kamaboko gel at various setting times and setting temperatures. **a** *Suwari* gel; **b** acid-induced kamaboko gel

related to the mechanical properties of the gel [23, 26, 27]. During setting, crosslinking of myosin was established and led to MHC band reduction and polymer band increase, as revealed by SDS-PAGE analysis. Comparing changes in breaking strength, which increases with setting time and temperature, to myosin heavy chain content, which decreases with setting time and temperature, suggests that lower myosin heavy chain content is associated with higher breaking strength in *suwari* gel and acid-induced kamaboko gel.

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