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# Effect of Garlic, Chili and Ginseng on the Thermal Gelation of Alaska Pollack Surimi

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**Abstract** Additions of ground garlic, chili and ginseng powder did not affect the breaking force and strain of directly heated gel of Alaska pollack surimi. In comparison, these additives reduced the setting effect achieved by incubation of the salted surimi at 25°C, and resulted in a decreased breaking force and strain for the two step heated gel. Garlic almost completely inhibited the myosin cross-linking reaction, an important reaction for improving the gel properties occurring in the setting process. However, chili and ginseng powder minimally inhibited the cross-linking reaction. Thus, this study proposes that the mechanism for the suppression of the setting effect by chili and ginseng is different from that of garlic.

**Keywords:** myosin, thermal gel, garlic, chili powder, ginseng

## Introduction

Korean people intake a substantial quantity of garlic and chili in various ways. These substances have been well established to have health promoting functions. Chili reduces fat storage (1) and garlic is known for its anti-thrombotic (2), antimicrobial (3, 4) and antihypertensive (5) actions. Ginseng is traditionally used as functional food as well as a drug. Promoting the intake of these substances by people in other countries requires the incorporation of these materials into well established food or into a newly development food that is easily consumed. One of the possible ways for the incorporation of garlic, chili and ginseng into food is through surimi based products because thermal gel formation is an excellent method to incorporate any kind of additive. Previously, we succeeded in the development of Kamaboko gel containing functional fish lipids: triglycerides and phospholipids (6). The thermal gelation of surimi is regarded as a controlled denaturation process of myosin; unfolding induced by heat is followed by a stable matrix formation (7). In Kamaboko processing in Japan, the step termed "suwari" or "setting", a procedure to keep the salted-surimi at a relatively low temperature prior to heating at a high temperature, is included for improving the gel strength of the final product. The potential action of transglutaminase (TGase, E.C. 2.3.2.13) catalyzing a cross-linking reaction of the myosin heavy chain (HC) in the setting process has been proposed (8, 9). In the current study, we developed a method to produce new functional Kamaboko made from Alaska pollack surimi containing garlic, chili or ginseng. We also studied how their additions affect the thermal gelation of surimi. Their effects on the breaking force and strain of the directly heated gel, and for the two step heated gel are separately discussed.

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## Materials and Methods

**Materials** Fresh garlic, chili powder, and powdered ginseng were all purchased in a local market in Pusan, Korea. Frozen surimi (FA grade) of Alaska pollack (*Theragra chalcogramma*) was donated from Nissui Co. Ltd. (Tokyo, Japan). The surimi contained 4% sucrose, 4% sorbitol and 0.25% sodium polyphosphate. The contents of the water and proteins of the surimi used were 75.3% and 17%, respectively.

**Preparation of thermal gel** Frozen surimi stored at -40 °C was transferred to a cold room (4°C) prior to use. Diced surimi was chopped with a food processor (Speed Cutter MK-K7, Matsushita Electric Industrial Co., Ltd. Osaka, Japan). To the surimi, 25% (w/w) cold water was added together with 2.5% (w/w) NaCl. The mixture was further chopped eight times for 30 sec each time with 30 sec intervals to make a homogeneous salted meat paste without elevating the temperature. Ground garlic, chili, and ginseng powders were successfully added homogeneously to the salted meat paste. The prepared salted paste containing the materials above was subsequently stuffed into an aluminum pipe (17 mm diameter and 20 mm long with a holding of about 4.5 g) as a container, and wrapped with polyvinylidene chloride film (Riken Vinyl Industry Ltd., Tokyo, Japan). All of the above procedures were conducted in a cold room at about 5°C. The samples were transferred to a water bath controlled at 25°C and kept for setting. The set gel was immediately transferred to a water bath at 90°C and cooked for 20 min to produce Kamaboko or thermal gel. Cooking was stopped by cooling the samples in ice, and stored in a cold room until rheological measurements were performed.

**Rheological measurement of thermal gel** Before the puncture test, the thermal gel was taken from the cold room and left for 2-3 hr at room temperature (about 25°C). The gel was then removed from the container carefully.

The breaking force (g) and deformation (strain) (mm) of the gel were both recorded on a Rheometer RE-3305 (Yamaden, Tokyo, Japan) using a 3 mm diameter cylindrical plunger with a loading speed of 0.5 mm/sec.

**Solubilization of the thermal gel and sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)** To study the cross-linking of myosin occurring in the setting process, the Kamaboko gel was dissolved in a solution containing 8M urea, 2% SDS, 2% 2-mercapto-ethanol, and 20 mM Tris-HCl (pH 8.0) by constant stirring overnight at room temperature (10). The solution for dissolving the Kamaboko gel was termed as SDS-U-M solution. The solution was clarified by centrifugation at 3,000 × g for 15 min, and the clear supernatant was saved as a solubilized protein solution. Practically no sediment was formed by the centrifugation process except the insoluble chili and ginseng added. To analyze the cross-linking reaction, the solubilized solution was applied to mini slab gels (8 × 9 cm). SDS-PAGE was carried out on 3% polyacrylamide - 0.5% agarose gel containing 0.1% SDS (10). The preparative method of the agarose-polyacrylamide gel was followed as described earlier (11).

The gel system allowed us to detect the cross-linked myosin heavy chain components in the pattern. After electrophoresis, protein bands were stained with Coomassie Brilliant Blue R-250. Acrylamide HG (Wako Pure Chemicals Industry Ltd. Osaka, Japan) and Agarose GP-36 (Nakalai Tesque, Kyoto, Japan), both of electrophoresis grade, were found suitable for preparing the above fragile gels.

## Results and Discussion

**Thermal gelation of surimi containing ground garlic** In order to characterize the effect of garlic on gel formation, it was essential to mix garlic into surimi homogeneously. Salt ground surimi was found to be the most suitable for a homogeneous mixing of ground garlic. Salted surimi with and without 3% ground garlic was preheated (set) at 25°C up to 4 hr and cooked at 90°C to produce the two-step heated gel. The breaking force and breaking strain (or deformation) of the two-step heated thermal gels are shown in Fig. 1A and B, respectively. In the absence of garlic, increases in the breaking force and strain during the 25°C preheating period before heating were clearly demonstrated. This phenomenon was referred to as the setting effect or Suwari. Preheating of the ground surimi for 4 hr increased the breaking force from 350 g to 1,000 g: roughly a 280% increase. Concurrently, the breaking strain increased from 14 mm to 17 mm. Thus the setting effect by preheating at a relatively low temperature was well recognizable with the surimi employed. When the ground garlic (3%) was added to the salted surimi, the increases in the breaking force and strain by preheating were significantly suppressed as demonstrated in Fig. 1A and 1B. For instance, setting for 4 hr very slightly increased the breaking force from 200 g to 270 g for the gel containing 3% garlic. The breaking strain by setting was also decreased by the addition of garlic. It should be noted that an addition of garlic did not affect the breaking force and strain for the directly heated gel (set time 0 hr). It was demonstrated that garlic decreased these two rheological parameters of the thermal gel by diminishing the setting effect. The inhibitory effect of garlic on setting was further studied by varying the amount of garlic added. Salted surimi containing various amounts of garlic up to 5% was preheated at 25°C for 4 hr and cooked at 90°C. Thermal gels without preheating were also prepared. The breaking forces and strains obtained are shown in Fig. 2A, B. The breaking force and strain for the directly heated gel without preheating were almost constant irrespective of the amount of ground garlic added, which is the same trend illustrated in Fig. 1. An incubation of the salted surimi for 4 hr increased the breaking force by about three times when the surimi contained no garlic (Fig. 2A). However, this high breaking force gradually decreased with increasing the amount of garlic added, and finally approached a level similar to that for the directly heated gel. Approximately 5% garlic was sufficient for a complete loss of the setting effect under the experimental conditions. It was, therefore, concluded that garlic reduced the gel strength of the thermal gel by suppressing the setting effect rather than by inhibiting heat-induced gel formation at high temperatures.

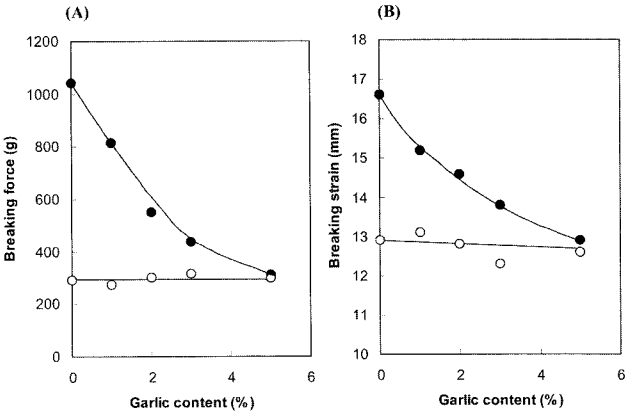


**Fig. 1. Effect of ground garlic on the thermal gelation of Alaska pollack surimi.** ( ● ), Gel without garlic; ( ○ ), gel with 3 % ground garlic. (A), Breaking force; (B), Breaking strain.

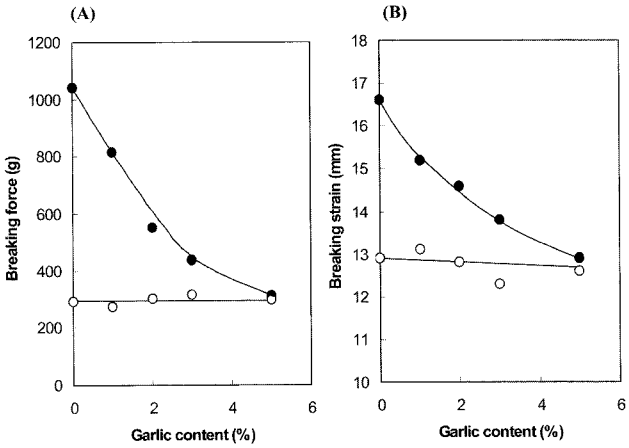


**Fig. 2. Ground garlic content dependent suppression of setting.** ( ○ ), Gels directly heated; ( ● ), Gels produced by two step heating (preheating time was 4 hr). (A), Breaking force; (B) Breaking strain.

**Myosin cross-linking reaction in the presence of garlic** The cross-linking reaction of myosin catalyzed by TGase is one of the prominent events detectable during the setting process. Cross-linked myosin is believed to be attributed to the network structure of set gel (8-10). Since the setting was drastically suppressed upon the addition of garlic, we examined whether garlic inhibited the cross-linking reaction of myosin. The myosin cross-linking reaction in the thermal gel was monitored on mini slab gels consisting of 3% polyacrylamide and 0.5% agarose in the presence of SDS. Agarose was added to strengthen the slab gels because of the low density of the polyacrylamide, which was essential for the separation of the cross-linked myosin heavy chain (HC) with a high molecular size on the gels. As shown in Fig. 3, the SDS-PAGE system resolved a high molecular weight band such as connectin with a mass of 2,000 kDa (12). The same gel system also resolved a relatively low molecular weight band around 20 kDa such as myosin light chain components and troponin subunits, although the resolution was poor. The resolution between the myosin HC and the actin band was quite good. Thus, this gel system seemed suitable for the analysis of the changes in protein composition of surimi, especially, the myosin HC during the setting process because the HC would be converted into either a cross-linked polymer catalyzed by TGase or a degraded fragment catalyzed by protease. The most dramatic change in the gel patterns was a decrease in the myosin HC content accompanied by a generation of cross-linked HCs denoted as HC2, HC3, HC4 and HC5 and polymers stacked on the tops of the gels (9). The stacked polymers might be highly cross-linked HCs unable to penetrate into the polyacrylamide gel network. The gel preheated for 5 hr contained practically no monomer HC in the pattern. A small increase in the density of the HC degraded 150 kDa band migrating below the myosin HC was detectable (13). When 3% garlic was added to the surimi gel, the decrease in myosin HC content during the preheating was remarkably suppressed. Although HC oligomers were detectable, the main product was HC2 (Fig. 3B). The amounts of remaining HC and the 150 kDa were estimated densitometrically and the results are presented in Fig. 4A, B. A decrease in HC content is caused by either cross-linking or degradation, thus the sum of these two components shows the myosin content uncross-linked in the surimi gel. When decrease in HC for the control gel was quick, namely cross-linking occurred quickly, and the remaining monomer for the 2 hr incubated gel was about 28%. The value for the gel containing garlic was still about 78% by 2 hr incubation. A slow decrease of the monomeric component in the garlic containing gel was probably due to the slow progress of cross-linking under the experimental conditions. These results demonstrate that the component contained in ground garlic has the ability to inhibit the cross-linking reaction catalyzed by TGase. Therefore, the suppression of the setting effect by garlic was the consequence of the inhibition of the cross-linking reaction catalyzed by TGase.

As the suppressing effect of garlic on setting was observed, we also examined the effect of commercially available dried garlic powder. The dried garlic powder showed a similar inhibitory effect as did ground garlic (data not shown). The component that attributed to the inhibition of myosin cross-linking and suppression of the setting effect seemed to be quite stable.

**Effect of chili powder on the thermal gelation of surimi** Another spice we studied was chili powder. The same set of experiments as performed with ground garlic was conducted. Ground dried chili powder was directly mixed with salted surimi. We were concerned whether the homogeneous mixing of chili powder to salted surimi was possible; however, the chili powder particles could not be detected in the final product of Kamaboko gel. But, the entire Kamaboko gel turned a red color. Therefore, we concluded that chili powder can be mixed quite homogenously with surimi. This was probably because we used finely ground chili powder. The change in the breaking

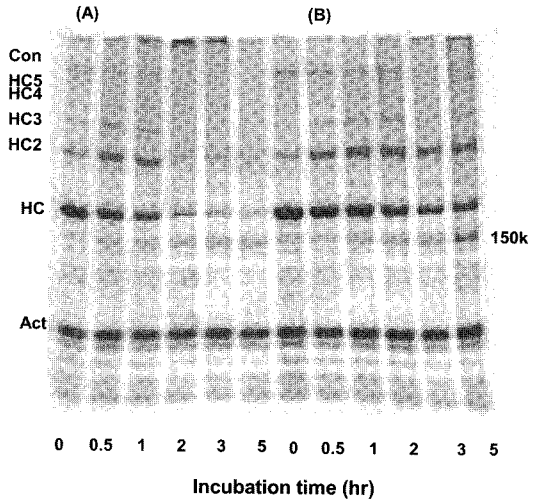


**Fig. 3. Change in the SDS-PAGE patterns of salted surimi during the setting with or without garlic.** (A), Gels without garlic; (B), Gels with 3% garlic. HC, HC2, HC3, HC4, and HC5 are a myosin heavy chain monomer, dimer, trimer, tetramer, and pentamer, respectively. Act, Con and 150k are actin, connectin bands and a 150 kDa fragment, respectively.

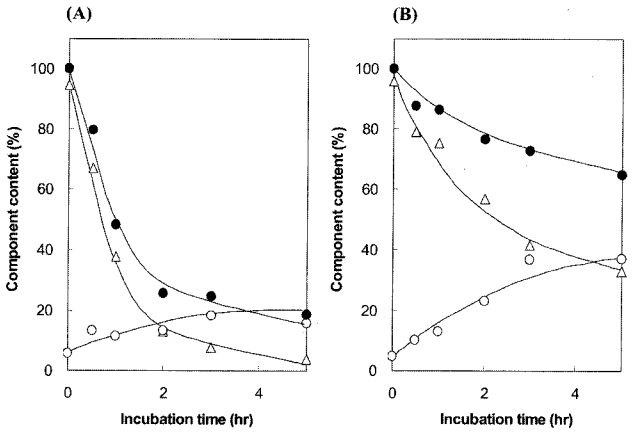


**Fig. 4. Estimation of the myosin monomer and 150 kDa fragment during preheating.** (A), Gels without garlic; (B), Gels with 3% garlic. A myosin HC monomer ( △ ), a 150 kDa fragment ( ○ ) and the sum of these two ( ● ) were estimated from the pattern presented in Fig. 3.

force during the setting at 25°C in the presence of 5% chili powder is shown in Fig. 5A. The increase in the breaking force was almost completely inhibited by the addition of chili powder. The increase in the breaking strain during the preheating is shown in Fig. 5B. The strain for the directly heated gel was remarkably decreased from 14.7 to 8.8 mm upon the addition of chili powder. The strain slightly increased upon preheating. These observations are somewhat different from those obtained using garlic. We further studied the concentration dependent inhibition by chili powder. The results are presented in Fig. 6A and B. The breaking force without preheating was scarcely affected by the chili added, while the breaking force for the gel set for 4 hr gradually decreased upon the addition of chili. The breaking strain for the directly heated gel at 90°C as well as the two step heated gel decreased with increased chili content. The effect of chili was therefore distinguished from that of garlic regarding the content dependency.

As the suppression of the setting effect by garlic was well explained by the inhibition of cross-linking, we analyzed the cross-linking reaction in the presence of chili on SDS-PAGE. Densitometric estimations of the myosin HC monomer and the 150 kDa degraded fragment are shown in Fig. 7. The decrease in myosin HC content upon preheating for the gel containing chili powder was slower than that without chili; however, the difference was remarkably small compared with the case of garlic. The densitometric estimation of HC demonstrated that the decrease in HC was very slightly slower in the presence of chili powder. Productions of the 150 kDa fragment were almost the same for the gels with or without chili powder. It was thus concluded that virtually all cross-linking of myosin was not inhibited by chili powder. Therefore, the suppression of setting by the chili powder addition was not due to the inhibition of the myosin cross-linking reaction. It is generally believed that the setting effect is always accompanied by TGase mediated myosin cross-linking (14). However, the present result showed that the setting effect could be lost without inhibition of TGase. Ground chili particles homogeneously distributed in the thermal gel removed the setting effect. As the addition of chili powder decreased the breaking strain for the directly heated gel, the gel network formed by myosin association rather than myosin cross-linking was inhibited by chili powder. Even though we employed finely ground chili powder, it did not dissolve in salted surimi because the protein solubilization with the SDS-U-M solution yielded the chili powder as sediment upon centrifugation. The presence of the fine chili powder particles in the set-heated gel may have damaged the network structure formed during the preheating process leading to the reduced breaking force and strain. All of the above results were obtained with dried chili powder. We further investigated the association of chili to changes in gelation using soaked dried chili powder. Dried chili powder was soaked in water overnight, and its effect on gelation was examined. However, the result was exactly the same as that achieved using dried chili powder. Namely, the addition of swollen chili powder inhibited the setting effect without inhibiting the myosin cross-linking reaction.

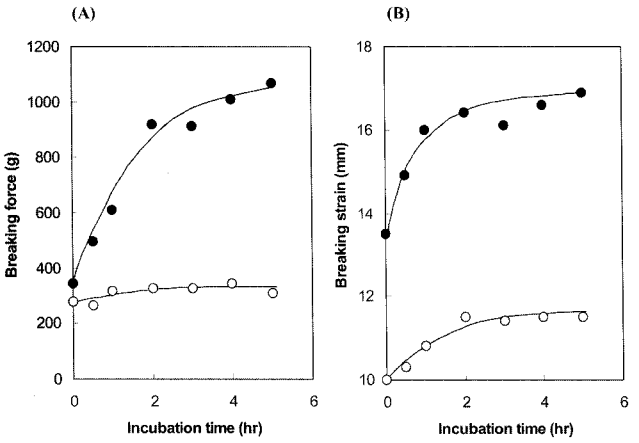


**Fig. 5. Effect of chili powder on the thermal gelation of Alaska pollack surimi.** ( ● ), Gel without chili; ( ○ ), Gel with 3% finely ground chili powder. (A), Breaking force; (B), Breaking strain.

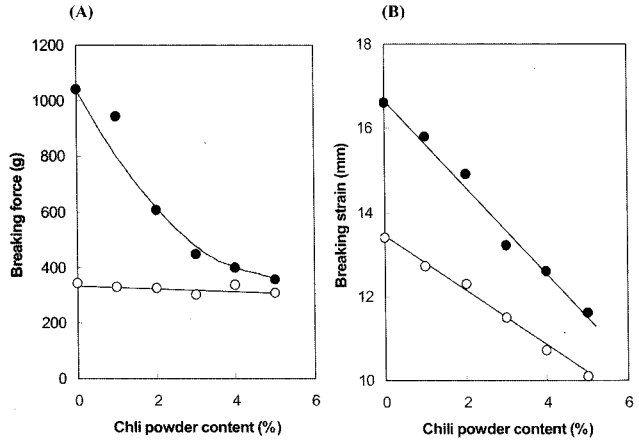


**Fig. 6. Chili powder content dependent suppression of setting.** (○), Gels directly heated; (●), Gels produced by two step heating (preheating time was 4 hr). (A), Breaking force; (B), Breaking strain.

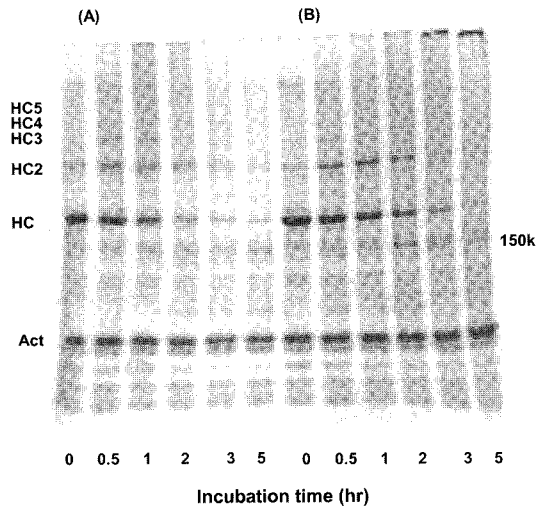


**Fig. 7. Myosin cross-linking in the presence of chili powder.** (A), Gels without chili powder; (B), Gels with 3% chili powder. The other conditions and the symbols were the same as in Fig. 3.

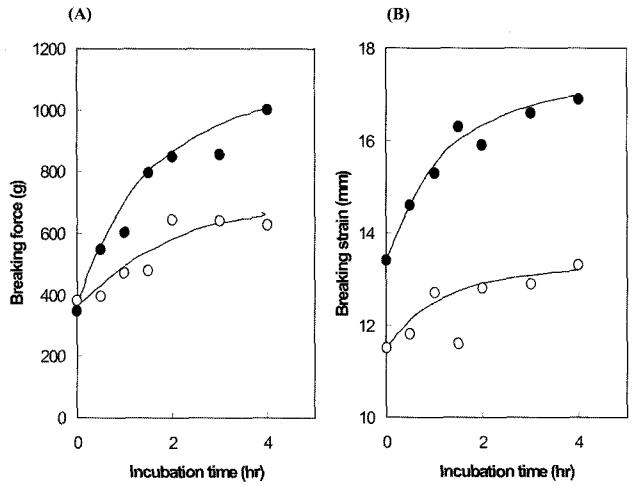


**Fig. 8. Effect of ginseng powder on the thermal gelation of Alaska pollack surimi.** (●), Gel without ginseng powder; (○), gel with 3% finely ground ginseng powder. (A), Breaking force; (B), Breaking strain.

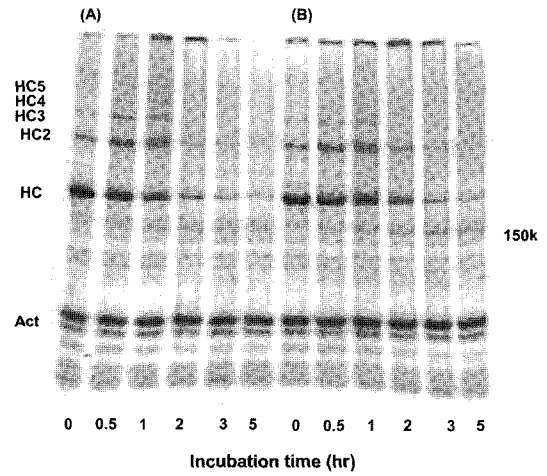


**Fig. 9. Myosin cross-linking in the presence of ginseng powder.** (A), Gels without ginseng powder; (B), Gels with 3% ginseng power. The other conditions and the symbols were the same as in Fig. 3.

**Effect of ginseng powder on the thermal gelation of surimi** We also examined the effect of dried ginseng powder on gelation. The content of ginseng employed was 3% (Fig. 8). Its effect was very similar to that of chili powder. Namely, ginseng powder reduced not only the breaking force but also the breaking strain achieved by setting; moreover, the breaking strain for the directly heated gel was reduced from 13.5 to 11.5 mm (Fig. 8). We also found that cross-linking was not inhibited by the addition of ginseng powder (Fig. 9). Ginseng powder and chili powder contain insoluble vegetable fiber. The changed structure of the surimi gel caused by the additives used in this study is a plausible reason for the reduction in gel properties. Especially, unknown components of garlic may have inhibited the myosin cross-linking reaction even though garlic contains vegetable fiber.

In this study, garlic, chili powder and powdered ginseng caused reductions in gel properties although their reducing mechanisms were different. We propose that if the amounts of additives were reduced to 0.5%, the breaking force and strain for the two step gel would be kept at about 90% of those of gel without these additives.

## Acknowledgments

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