Presence of Endopeptidase in the Powdered Yam *Dioscorea opposita* and Its Effect on the Texture of Kamaboko

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Yam is often added to kamaboko, such as Hanpen and Shinjo. We investigated the presence of endopeptidase in powdered yam and the effect of its addition on the texture of kamaboko. Hydrolyzing activities of casein and myofibrillar protein were observed in a crude extract of powdered yam. In addition, the optimum temperature and pH against casein and myofibrillar protein were approximately 70°C and 7.4, and 60°C and 6.8, respectively. Approximately 60% of the endopeptidase activity remained even with 3% NaCl. In the kamaboko to which powdered yam had been added degradation of the myosine heavy chain and decrease of the elasticity were observed.

Keywords: kamaboko, yam, endopeptidase, Alaska pollack

There are many of kamaboko in Japan. One major reason why there is so much variation is that kamaboko is prepared from many kinds of fish species such as Alaska pollack, pike eel, threadfin beam, and white croaker, and each fish has an individual flavor and various gel forming properties. Another reason is that there is no limit to the other materials that can be added to kamaboko. Many varieties of vegetables, dairy products and meat products, and ingredients like seasonings and emulsifiers contribute to the wide variation in kamaboko. Some sub-materials added to kamaboko, however, are known have endopeptidase activities and the endopeptidases sometimes hydrolyze myofibrillar proteins, which form an elastic network structure in the finished product (Sakasai *et al.*, 1980; Makinodan *et al.*, 1990). For example, ginger, one ingredient often added, shows endopeptidase activity, and causes degradation of the myosine heavy chain (MHC) and decrease in the gel strength of kamaboko (Makinodan *et al.*, 1990). Yam, *Dioscorea opposita*, is also added to kamaboko, such as in traditional types of Hanpen and Shinjo. Characteristics of these products are a soft texture produced by the formability and viscosity of yam. If yam has endopeptidase, as ginger does, its addition will affect the gel strength of kamaboko. The effect of yam addition on the gelling properties of kamaboko has not yet been investigated, however.

In this study, we attempted to determine the existence of endopeptidase in powdered yam (PY). We also studied the degradation of MHC caused by endopeptidase and the effect of PY addition on the texture of kamaboko.

Materials and Methods

Preparation of crude extract from powdered yam PY, which was supplied by Senba Touka Kogyo (Tokyo), was dissolved in 12 volumes of distilled water and stirred at 4°C overnight. The solution was then centrifuged at 10,000×

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Table 1. Evaluation criteria of folding test.

<table>
<thead>
<tr>
<th>Score</th>
<th>Criterion</th>
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</thead>
<tbody>
<tr>
<td>5</td>
<td>no crack when folded in four</td>
</tr>
<tr>
<td>4</td>
<td>no crack when folded in two</td>
</tr>
<tr>
<td>3</td>
<td>gradually cracked by folding in two</td>
</tr>
<tr>
<td>2</td>
<td>immediately cracked by folding in two</td>
</tr>
<tr>
<td>1</td>
<td>mud like</td>
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</table>

Results and Discussion

Casein hydrolyzing activity of crude extract. The endopeptidase activity of the crude extract was determined with casein as a substrate. Figure 1 shows the effect of temperature in the neutral pH range in which kamaboko is usually prepared. A linear increase of the hydrolyzed product, TCA-soluble materials, was observed for 30 min from 40°C to 70°C, but little hydrolyzed product was observed at 80°C. Therefore, endopeptidase activity was proved to be present in the crude extract of PY. This activity showed an optimum temperature around 70°C. The pH effect on the endopeptidase activity of the crude extract was determined and is shown in Fig. 2. The optimum pH of the crude extract was approximately 7.4 at 60°C for 30 min.

Effect of NaCl on endopeptidase activity of crude extract. Approximately 2% NaCl is usually added to kamaboko. Some proteases lose their activity in high NaCl solutions; therefore we measured the effect of NaCl at 1%, 2%, 3%, 5% and 10% final concentration, on the endopeptidase activity of the crude extract. As shown in Fig. 3, casein hydrolyzing activity decreased which increase in NaCl concentration. However, about 60% of the endopeptidase activity remained at a 3% NaCl condition, which is the ordinary NaCl concentration in kamaboko.

Myofibrillar protein hydrolyzing activity of crude extract. Casein hydrolyzing activity was observed even in the presence of NaCl. Next, we measured the myofibrillar protein hydrolyzing activity of the crude extract. Changes in the hydrolyzed product at a temperature of from 40°C to 80°C are shown in Fig. 4. Linear increases of the hydrolyzed product were observed for up to 90 min below 60°C. At above 70°C, the linearity between hydrolyzed product and incubation time was not observed, and there was little increase in the hydrolyzed product. The optimum temperature of activity in the crude extract was approximately 60°C, which was lower than that with casein substrate. The difference of optimum temperature between casein and myofibrillar protein substrates is assumed to be due to the difference in their heat treatments during preparation. Casein had a heat-denatured treatment when it was used for substrate. On the other hand, myofibrillar protein had not. Therefore, a conformation change of myofibrillar protein during the reaction may affect the hydrolyzing activity of the crude extract. We did not confirm the pH in the reaction mixture at each temperature. It is also possible that pHs in reaction mixtures differed at 70°C and affected the hydrolyzing activity of the crude extract.

Then, we measured the pH effect on the myofibrillar protein hydrolyzing activity of the crude extract (Fig. 5). The optimum pH for such activity was around 6.8 at 60°C for 30 min. This is 0.6 of a point lower in pH than that with the casein substrate. Since the optimum temperature of the crude extract was considerably high, we measured its thermos-
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Fig. 4. Effect of temperature on the myofibrillar protein hydrolyzing activity of the crude extract from powdered yam. Myofibrillar protein hydrolyzing activity was measured at pH 7.0; ○, 40°C; ●, 50°C; △, 60°C; ▲, 70°C; ■, 80°C.

Fig. 5. Effect of pH on the myofibrillar protein hydrolyzing activity of the crude extract from powdered yam. Myofibrillar protein hydrolyzing activity was measured at 60°C for 30 min. ○, KH₂PO₄-Na₂HPO₄ buffer; ●, Na₂CO₃-NaHCO₃ buffer.

Fig. 6. Heat-stability of myofibrillar protein hydrolyzing activity of the crude extract from powdered yam. Myofibrillar protein hydrolyzing activity was measured at pH 6.8 at 60°C for 30 min after heating at various temperatures up to 30 min. ○, 60°C; ●, 65°C; △, 70°C; ▲, 75°C; ■, 80°C.

Table 2. Result of folding test on kamaboko.

<table>
<thead>
<tr>
<th>Heating time (min)</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>60°C Without PY</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>With PY</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>85°C Without PY</td>
<td>3</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>With PY</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Fig. 7. SDS-PAGE analysis of kamaboko prepared with or without powdered yam. Arrow shows the position of 130 k. M, maker; HC, myosin heavy chain; A, actin; a, c, and e, without powdered yam; b, d, and f, with powdered yam; BH, before heat; 60°C, 60°C heating; 85°C, 85°C heating.

tability. First, the crude extract was heated at pH 6.8 at 60°, 65°, 70°, 75° and 80°C for 10, 20 and 30 min and then immediately cooled in an ice bath. Second, the endopeptidase activities were measured at 60°C; as shown in Fig. 6, this endopeptidase was stable at 60°C for 30 min. However, when the temperature rose above 65°C, the endopeptidase activity dropped remarkably. The remaining activities at 70°C and 80°C for 10 min were 55% and 0%, respectively.

Physical property of kamaboko added with powdered yam. Since the PY endopeptidase showed myofibrillar protein hydrolyzing activity even in the presence of NaCl at neutral pH, we examined the effect of added PY on the physical property of kamaboko. The results of the folding test are shown in Table 2. Scores of the kamaboko with or without PY heated at 60°C were lower than those at 85°C, and kamaboko prepared with PY was weaker than at without PY at both temperatures. Degradation of kamaboko at 60°C seemed to be a modori phenomenon, and further degradation of kamaboko with PY seemed to reflect the effect of PY endopeptidase. However, kamaboko with PY was weaker than that without PY at 85°C, which seemed to be the same effect as at 60°C. Since scores of the folding test of kamaboko at 85°C rose for up to 90 min, heating-time dependent degradation did not occur in kamaboko at 85°C. As shown in Figs. 1 and 4, PY has little endopeptidase activity at 85°C. Endopeptidase activity may have caused the degradation of protein under rising temperature, or the PY viscosity may affect the physical properties of kamaboko at 85°C.

An SDS-PAGE analysis of kamaboko was performed to determine whether myofibril degradation occurred at various
temperatures. SDS-PAGE patterns of kamabokos with or without PY heated at 60°C and 85°C are shown in Fig. 7. In both kamaboko samples, the density of MHC decreased and that of some new bands, which migrated around 150 k and below 43 k, increased with heating at 60°C. The degradation magnitude of MHC in kamaboko with PY was greater than that without PY. The degradation of MHC in kamaboko without PY was suggested to be a modori phenomenon caused by endogenous endopeptidases such as heat-stable alkaline proteinase (Makinodan et al., 1987) and trypsin-like heat-activated serine protease (Toyohara & Shimizu, 1988; Kinoshita et al., 1992). Therefore, these changes in kamaboko heated at 60°C with PY are assumed to be affected by the endopeptidase present in PY as well as by endopeptidases which induced modori. The degradation of MHC and formation of the 150 k band of both kamaboko samples heated at 85°C were less than those at 60°C. Furthermore, there was little difference in the SDS-PAGE patterns of kamaboko heated at 85°C for 30 min and 60 min (data not shown). These changes thus seemed to occur with rising temperature, and to determine what caused them. It seemed necessary to devise a form of kamaboko or heating method.

We used lyophilized powdered yam in this study which is often used for processed food instead of raw yams because of its convenience, availability and price stability. Arai et al. (1996) investigated the rheological properties of raw and lyophilized yams and found the lyophilized powdered yam had lower values of initial viscosity and maximum viscosity than raw yam according to dynamic viscoelasticity measurements. Therefore, the endopeptidase activity of raw yam may be higher than that of lyophilized yam. In any case, we should remember the endopeptidase activity of yam when we add yam to food containing protein.

References