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Regulation of squid liver protease by the combination of salt and minerals

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Summary

We utilize only edible muscle part of squid as food. Viscera are discarded as an industrial waste. It is well known that squid liver contains various types of protease activity. The purpose of the research is to characterize the degrading ability of the protease in squid liver aiming to utilize them as useful bioactive enzymes. Muscle protein of fish (myosin) was used as a substrate for the enzymes. Cleavage pattern of myosin by the enzymes were studied by changing the digestion conditions. Lipid binding ability of the digestion products was measured to test the possibility as the food functional additives.

Squid liver contains three types of protease, cystein- (CP), metallo- (MP), and serine-protease (SP) as revealed by the inhibition test. A mixture of three inhibitors for the respective enzymes completely inhibited the digestion by the crude enzyme. Digestion pattern for each enzyme was studied by eliminating one out of the three inhibitors. MP showed the highest activity cleaving myosin at near center. CP was less active than MP. Digestion patter for CP was less specific producing enormous numbers of short peptides. Activity of SP was the lowest among three enzymes. Cleavage site by these three enzymes were studied by using ammonium fractionation and Western-blotting method of the products. All of enzymes preferentially cleaved roughly at the center of myosin molecule in an early phase. Elevation of the digestion temperature to 37℃ promoted the digestion by CP. Contribution of MP became less at high temperature. Consequently, major products at high temperature were short peptides mainly produced by CP.

Effect of salts (KCl, NaCl) on the activity for the three enzymes was further studied. Activity at 0.1 M was low, while increased with increasing salt concentration to 0.3 M-0.5 M when crude enzyme was used. The concentration was one for dissolving myosin by salts. A further increment of salt concentration rather inhibited the activity. CP and MP both showed a high activity in the presence of NaCl. KCl inhibited the activity of CP, while the activity of MP was not. Increase in neither NaCl nor KCl concentration increased the activity of SP. These results suggested that the size of the products would be regulated by controlling the type of salts and their concentrations considering the properties of three enzymes.

Lipid binding abilities of degraded myofibril proteins with different sizes were studied to examine the possibility to use as functional food additives. Irrespective of the sizes of the products, both hydrolyzed products bind soybean oil. The product with long peptides was superior to one with short peptides. The ability was much better than that of egg white.