## Effect of Salt-Water Treatment on Black-Spot Formation of Shrimp

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## Summary

Melanization in crustaceans is a critical problem in the preservation and processing of shrimp and crabs. It causes the deterioration of food value by forming black spots on the body after harvest and food distribution. Generally, mellanization in crustacean is mainly caused by the oxidation reaction by phenoloxidase (PO). Crustacean PO is classified as type 3 copper proteins. PO catalyzes the oxidation of mono- and di-phenol compounds, which is the rate-limiting step of melanization. Similarly, food browning is also a crucial problem on food preservation of vegetables and fruits. The reaction is catalyzed by polyphenoloxidase (PPO) that is also copper-containing protein of which catalytic center has similar structure to that of crustacean PO. To prevent the browning on a section of fruit, salt water treatment is generally used. In this study, the author attempted to prevent mellanization of crustacean by pre-treating shrimps with various concentrations of salt-water. However, the treatment was not effective on prevention of mellanization in freeze-thaw process of shrimp.

The mellanization was especially severe in drip from the thawed shrimp. Thus, the author investigated the main source of PO activity in hemolymph of the shrimp. In hemolymph of crustacean, there is an abundant protein hemocyanin (Hc) that generally functions as a dioxygen-transporting protein. To date, many studies have shown PO activity in Hc. Hence, the author re-examined the source of PO activity in hemolymph by focusing on the purification method of Hc and hemolymph-type PO. The conventional procedure for the preparation of arthropod Hc, which includes precipitation of Hc by ultracentrifugation and subsequent purification by size exclusion chromatography, was not able to remove hemolymph-type PO from Hc, completely. In contrast, fractionation with 50% saturation of ammonium sulfate and subsequent hydrophobic chromatography yielded sufficiently pure Hc, which contained no detectable PO. The Hc preparation without PO had no detectable PO enzymatic activity under the experimental conditions employed in this study. These results indicate that the main source of PO activity in the hemolymph of kuruma prawn is hemolymph-type PO and that the conventional purification method of Hc is insufficient for evaluating the PO activity of Hc..