カニ缶詰肉におけるブルー・ミートの発生原因およびその機構 V

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A Cause and Mechanism of Blue Discoloration of Canned Crab Meat—V. Isolation of Causative Substance of Blue Meat

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Although haemocyanin and hydrogen sulphide are involved in the blue discoloration of canned crab meat,[1] processing studies in progress in this laboratory have created a need for more detailed knowledge of the causative substance.

This work was undertaken to isolate it and study the chemical binding of copper and sulphide in the blue substance.

Materials and Methods

Blue and normal meats in commercially canned king crab were separated as thoroughly as possible. Pigments in the epidermis were extracted with 95% ethanol for 24 hrs. The blue meat was dried under vacuum and ground in a mortar. The blue substance was separated from the dry powdered blue meat as follows. A suspension was prepared by mixing 15.8 g of the dried blue meat with 350 ml of distilled water and 0.63 g of protease (Prozyme B, Kyowa Hakko Kogyo Co., Ltd.). After incubation at 40°C for 24 or 48 hrs., the suspension was diluted with an equal volume of 10% trichloroacetic acid, and centrifuged at 1,500×g for 20 min. The precipitate was stirred with 50 ml of distilled water for 5 min., and again centrifuged at 1,500×g for 20 min. This process of precipitation and dissolution was repeated three times. The precipitate was then washed with 20 ml of 95% ethanol for 5 min. and the alcoholic solution was discarded after centrifugation at 1,500×g for 20 min. The washing and precipitation was repeated five times until the precipitate formed a dark green-coloured crystal. The crystal was treated with chloroform-ethanol (30:7), dried under vacuum and used in all subsequent work. The yield of blue substance by this procedure was about 4.56%. Inorganic sulphide content was determined by a modification of the method of LOVENBERG, BUCHANAN and RABINOWITZ.[2] Three mg of sample was weighed in a stoppered test tube and mixed with 5 ml of 1% zinc acetate and 0.2 ml of 12% sodium hydroxide. The mixture was allowed to stand for 20 min. One ml of 0.5% N,N′-dimethylphenylenediamine hydrochloride and 0.2 ml of 0.023 M ferric chloride were added to the suspension. After 1 hr., the suspension was diluted to

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10 ml and centrifuged at 1,500×g for 20 min. The inorganic sulphide content of the supernatant was determined at 670 mμ with a Hitachi Perkin-Elmer UV–VIS spectrophotometer. Total nitrogen, iron, and copper contents were determined by Kjeldahl, o-phenanthroline,[3] and A.O.A.C.[4] methods respectively.

Results and Discussion

The chemical composition of blue substance I was similar to that of haemocyanin (Table 1). The photomicrograph of the blue substance I illustrated in Fig. 1–A seems to resemble heat-coagulated haemocyanin (Fig. 1–B). This finding suggests that blue substance I was derived from haemocyanin. In Table 2, the amounts of total nitrogen and

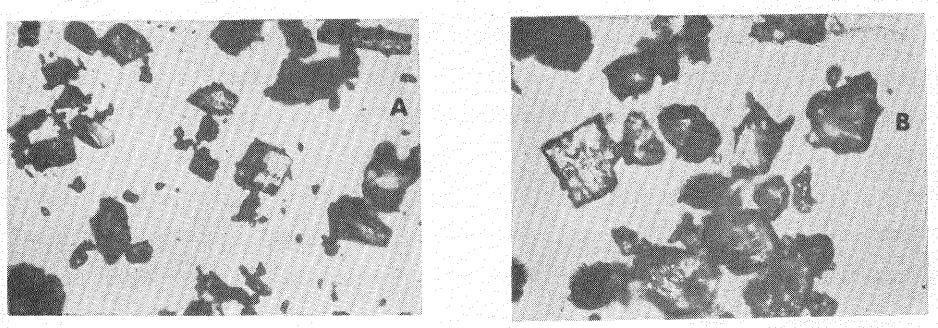

Fig. 1. Photomicrographs of blue substance I isolated from the blue meat of canned king crab (A) and heat-coagulated haemocyanin prepared from king crab haemocyanin (B).

Table 1. Analytical data of blue substance and haemocyanin of king crab, *Paralithodes camtschatica*.

	Blue substance I[1]	Haemocyanin
Copper (μg/mg)	1.49	1.20
Total nitrogen (mg/mg)	0.10	0.11
Crude protein (mg/mg)	0.63	0.69
Iron (μg/mg)	0.0	0.0
Inorganic sulphide (μg/mg)	0.0	0.0

[1] Incubated blue meat with protease at 40°C for 24 hrs.

Table 2. The purification of blue substance and its analytical data.

	Total nitrogen (mg/mg)	Copper (μg/mg)	Inorganic sulphide (μg/mg)
Blue meat	0.14	0.27	0.0
Blue substance I[1]	0.10	1.49	0.0
Blue substance II[2]	0.11	3.94	1.07
Blue substance III[3]	0.09	5.00	0.37

[1] Incubated blue meat with protease at 40°C for 24 hrs.
[2] Incubated blue meat with protease at 40°C for 48 hrs.
[3] Incubated blue substance II with protease at 40°C for further 24 hrs.

copper in 1 mg of the blue substance I are respectively less and more than those in the blue meat. This fact might be due to removal of impurities contained in the blue meat.

It has been reported that sulphide contained in organic materials such as cysteine, oxidized or reduced glutathione, insulin, and bovine serum albumin is not estimated by the method by LOVENBERG, BUCHANAN and RABINOWITZ.[2] Sulphide bound with iron in artificial non-heme iron proteins derived from bovine serum albumin (protein-iron-sulphide complex) and ferredoxin was measured by MCCARTHY and LOVENBERG[5] and LOVENBERG, BUCHANAN and RABINOWITZ.[2] Therefore, sulphide in haemocyanin may be different from that in protein-iron-sulphide complex and ferredoxin in respect to its mode of binding. The sulphide in the blue substance I could be measured after incubation for 48 hrs. at 40°C by the same method. This fact suggests that the sulphide in the blue substance II might become detectable after some breakdown of the protein. The reduction in sulphide in blue substance III may be due to its release during the enzymatic process. Thus it is considered that the blue substance I is the cause of the blue discoloration and is a sulphide complex which cannot properly be measured by the method by LOVENBERG, BUCHANAN and RABINOWITZ.[6] TAKAYASU and FUKUHARA[2] have investigated the amount of hydrogen sulphide evolved from blue and normal meats by ALMY's method,[7] and reported that there was a slight difference in the amount of it between them.

It has been presumed that haemocyanin would associated with the blue discoloration of canned crab meat. However, the chemical composition of the blue substance had not been ascertained sufficiently. This may be due to a difficulty of analytical methods to determine sulphide which combines strongly with copper.

Summary

The blue meat of canned crab was digested by protease, and a blue substance was isolated. The chemical composition of the substance was in accord with that of king crab haemocyanin-sulphide complex, apart from the S content. Since sulphide in the substance was not measured before protease treatment, but was detectable after prolonged enzymatic treatment (48 hrs. at 40°C), it was suggested that in fact the causative substance of the blue discoloration of canned crab meat was haemocyanin-sulphide complex.

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