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北海道大学水産学部研究彙報
CURD IN CANNED SALMON
II. Heat Coagulation of Drip and Flesh Extractives

Minoru Akiba*, Kanemichi Yamaguchi** and Michiaki Suzuki***

Introduction

As was observed in the previous paper (Akiba et al., 1968), curd from frozen salmon flesh occurred at two temperature ranges; one from 40° to 60°C, and another at about 80°C. This suggests there are two groups of heat coagulable proteins in salmon flesh. More amount of curd is formed in frozen salmon than in raw salmon unless completely thawed material is used. Therefore, it is supposed that the drip in frozen fish has a close relation to the curd formation, confirming earlier observations made by Konno et al. (1953), and by Stansby & Dassow (1951). Concerning the origin of drip in frozen fish flesh, the most evidence would suggest that drip is due to denaturation of proteins which normally tend to hold the water of the flesh and the changes in the cellular structure of the flesh brought about by the formation of ice (Tarr, 1942; Empey and Howard, 1954; Seagran, 1956; Tanikawa et al., 1963). Seagran (1958) had analyzed electrophoretically the protein constituents of drip from thawed fish flesh (yellow-stripped rockfish). He suggested that the sarcoplasmic protein fraction of fish flesh is not intimately associated with the origin of drip, and that the origin may be at least partially attributed to actomyosin denaturation by a dehydration process during freezing storage. The present paper reports informations of heat coagulation of drip in frozen salmon and flesh extractives from raw and frozen salmons.

Experimental

Heat coagulated matters in the extractives. Back flesh from raw and from frozen chum salmon which has been frozen for 24 weeks at -25°C was blended for 10 min with 4 times volume of distilled water or 15% sodium chloride solution for extraction. Solid portions were centrifuged (3000 rpm), and each 10 ml
of supernatant was poured into test tubes. After heating the test tubes on a water bath from 40° to 100°C at 10°C interval, coagulated matters were filtered, washed with distilled water, and dried in an oven at 95~100°C, then weighed.

**Heat coagulation of drip.** Chum salamon was stored for 30 weeks at -25°C and the back flesh was half-thawed at 0~1°C in a room for 8 hrs. The half-thawed flesh was wrapped in gauze and pressed under hydraulic pressure at 10 kg/cm². The exuded drip was subjected to centrifuge for 20 min at 3000 rpm, then each 2.5 ml of supernatant was poured into test tubes with 50 ml of 5% sodium chloride solution. The test tubes were heated from 40° to 100°C at interval of 10°C. Coagulation formed in the test tubes was separated by filtration, and was hydrolyzed with sulfuric acid for the determination of nitrogen contents. By the multiplication of 6.25 to the per cents of nitrogen, the curd percentage was obtained.

Variation in the amount of drip and its heat coagulation during freezing storage. Chum salmon was stored for 5 weeks at -25°C. At definite intervals, a portion (100 g) of the back flesh was cut off from the frozen salmon. The flesh was half-thawed and pressed at 10 kg/cm², then the volume of exuded drip was measured. The drip was centrifuged, then each 2.5 ml of the supernatant was poured into two test tubes with 50 ml of 5% sodium chloride solution. One tube was heated at 40°C and another at 100°C for 20 min, respectively. The coagulated matters were separated by filtration, and were used to determine the nitrogen contents.

Variation of heat coagulation of extractives during freezing storage. Minced chum salmon flesh was added to 4 times its volume of water or to 8 times its volume with 5% sodium chloride solution, and was homogenized by a waring blender for 40 min. Extractives obtained by centrifuging the homogenate were poured into test tubes up to 10 ml, frozen and stored at -25°C. The frozen extractives were thawed at 2~3°C after definite storing periods of 3 and 6 weeks. The variation of heat coagulation at a temperature from 30° to 100°C was determined by the same procedure as described above.

Results and Discussion

**Heat coagulation in extractives from raw and frozen salmons.** The amounts of heat coagulated matter in extractives from raw salmon flesh are shown in Fig. 1. Although the variation depends on temperatures and used extractants, a maximum amount was observed at 70°C and 50°C in a water and 15% sodium chloride solution extraction, at which temperatures the amount shows 0.66% and 1.4%, respectively. As it was also showed in the previous paper (Akiba, et al. 1968), because of the rapid coagulation of the surface layer of the flesh preventing internal fluid from coming out, the amount of curd at a higher temperature is
Fig. 1. The amounts of coagulated matter in extractives from raw salmon flesh. 
A: 15% NaCl soln. extraction (O), B: Water extraction (△), (A-B): Difference between A and B (×).

smaller than that at lower one.

In the case of an extraction with a 15% sodium chloride solution (A curve in Fig. 1), two coagulation temperatures were observed at 50°C and 80°C, showing at the former temperature more amounts of coagulation than that at the latter. While, in the water extraction, though it was also fairly observed, two coagulated matters clotted at below 60°C and above 70°C, the amounts of the former being less than that of the latter (B curve).

Provided that all proteins to be extracted by water are as well extracted by sodium chloride extraction, differences in the amounts of heat coagulation between the two extracted solutions may show the presence of two respective groups of coagulable proteins which they coagulate at about 50°C and 80°C in salt solutions (A–B curve in Fig. 1).

As shown in Fig. 2, similar results were obtained in extractives from frozen salmon flesh. The maximum amounts of clot by heating, however, were 1.57% at 70°C and 3.3% at 60°C by water and sodium chloride solution, respectively, which were always higher than those from raw salmon flesh. From the differences between the two extracted solutions, the presence of two kinds of coagulables at 50–60°C and 80°C is also presumed in the extractives by salt solutions, as is seen in Fig. 1. Such a difference of heat coagulation between water and salt solution has already been recognized in several kinds of fish extractives, e.g., by Simidu (1943) on snake-head, Takagi (1950) on flat fish and squid, and Tanikawa (1955) on sea cucumber.

Soluble proteins in fish flesh mainly consist of sarcoplasmic and myofibril proteins of which coagulation temperatures are generally 60°C–70°C and 35°C–45°C,
respectively (Mori and Asakawa, 1943; Simidu, 1943; Suyama, 1952; Tanikawa, 1955). Both sarcoplasmic and myofibril proteins are able to dissolve into salt solution. Therefore, in point of view of coagulation temperatures, two steps on the curve of heat coagulation might be observed as shown in Figs. 1 and 2.

**Heat coagulation of drip.** Exuded fluid from frozen salmon contained about 13% of the heat coagulable matter at 100°C, as shown in Fig. 3. A step of heat coagulation was observed from 40° to 60°C, and from 80° to 100°C. Percentages of coagulated matter were 27% and 80% at 40°C and 60°C heatings against that at 100°C heating. Thus, most part of proteins in the drip may be coagulated by heating under 60°C.

Seagran (1958) has determined the possible presence of the contractile
protein actomyosin of the myofibril in drip from frozen yellow-stripped rockfish, and showed the absence of actomyosin in drip. He has also analyzed the protein constituents of the centrifuged drip, and seven components which may be divided into three mobility groups by paper-electrophoresis, are established in the drip. Hence, it is supposed that the heat coagulation observed in Fig. 3, is mainly brought about by the presence of sarcoplasmic proteins, though chemical constituents of the drip in the experiment were not ascertained, and may be necessary for further experiments.

Variation in the amount of drip and its heat coagulation. As seen in Fig. 4, the amounts of exuded fluid were about 11% in the case of the raw salmon, but it amounted to about 20% after one week-freezing storage, and increased thereafter to 20~40% during 1~5 week-storage. At the same time, the amounts of heat coagulable at 100°C in the drip were increased from about 7 to 12%, and that amount at 40°C heating was also increased to about 2~4% during the freezing storage. The percentages of heat coagulable at 40°C were 24~32% against to that heated at 100°C which showed a slight increase throughout the storage. From the amounts of drip and those of coagulables at 100°C in the drip, the latter is calculated as about 0.8% in the raw flesh and 2.7% in the frozen flesh after 5 week-storage.

![Graph showing variation in the amounts of drip and its heat coagulation during freezing storage](image)

Fig. 4. Variation in the amounts of drip and its heat coagulation during freezing storage

○... Exuded drip, ×... Heat coagulated matter at 100°C, △... Heat coagulated matter at 40°C.

Assuming that the amounts of the clot formed on the surface flesh as curd at 100°C heating is about 30% to the total coagulated matter, (this was confirmed
in later experiments which will be described in a future publication), the yields of the curd are amounted as about 0.24% to the raw fish and 0.8% to the frozen fish, and those amounts are acceptable in practical cases as was shown in the earlier experiments (Akiba et al., 1968). Accordingly, it may be concluded that the presence of drip in the tissue has an intimate relation with the curd formation from frozen materials.

Variation of heat coagulable matters in extractives during freezing storage. The histograms of the ratios of coagulated matters at each heating temperature to the total proteins in the extractives are shown in Figs. 5 and 6. In Fig. 5, though most of the proteins were coagulated at 50°~60°C in the water extractives which were obtained from raw salmon, they were coagulated at 40°~50°C, and 30°~40°C in the extractives from 3 to 6 week-freezing storage, respectively. Thus, it is notable that heat coagulation temperature decreases

Fig. 5. Changes in the amounts of heat coagulable matter in water extractives during freezing storage
(1) Original extractives, (2) After 3 week-freezing storage, (3) After 6 week-freezing storage, A: Non-coagulable matter, B: Insoluble matter

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with elongation of the period of freezing storage. In the case of the 5% sodium chloride solution extractives the amounts of heat coagulated matter decreased during freezing storage, while the insoluble matters increased as shown in Fig. 6. Heat coagulation was mainly obtained by heating every sample at 20°~40°C. Comparing the results with Figs. 5 and 6, coagulation temperatures of the main component of heat coagulables were obviously different between the water and the salt solution extractives. The reason may be that a contractile protein, actomyosin, is extracted with 5% sodium chloride solution and coagulated by heating at 30°~40°C, while sarcoplasmic protein, myogen and others, is extracted with water and mainly coagulated by heating at 50°~60°C.

The results obtained about these extractives can not be applied directly to the native drip in frozen materials, so further experiments of the variation of

![Fig. 6. Changes in the amounts of heat coagulated matter in 5% NaCl soln. extractives during freezing storage](image)

(1) Original extractives, (2) After 3 week-freezing storage, (3) After 6 week-freezing storage, A: Non-coagulable matter, B: Insoluble matter

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heat coagulation on drip during freezing storage should be investigated.

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Summary

Heat coagulation of drip from frozen chum salmon and of the flesh extractives with water and salt solution was determined. The results obtained are summarized as follows:

1) Two coagulation temperatures were observed at about 50° and 80°C in the extractives.
2) The amounts of heat coagulation of extractives from frozen salmon were higher than those from raw salmon.
3) The heat coagulation of drip from frozen salmon was almost similar to those of extractives from raw and frozen salmons, and two groups of heat coagulable proteins in drip as a boundary were found at a temperature of about 60°C.
4) The heat coagulable temperature of extractive protein was tend to decrease together with the elongation of freezing storage.

References

Seagran, H.L. (1958). Analysis of the protein constituents of drip from thawed fish muscle. Food Res. 23(2), 143.