Changes in Gelatin in Hot Water Extracts of Snapping Turtle during Heating

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The changes in content and molecular weight of gelatin in hot water extracts from snapping turtles during heating were investigated. The amounts of gelatin and total nitrogen increased rapidly and reached a maximal value after 24 h of heating. The breakdown of gelatin molecules occurred during heating; the average molecular weights estimated by a gel filtration and an SDS-polyacrylamide gel electrophoresis were approximately 50,000 and 35,000 after 8 h, 28,000 after 12 h, 8,000 after 24 h, and 4,000 after 48 h. It was suggested that the degradation products of gelatin play an important role on the taste of the soup.

In the preceding paper,1) we examined the distribution of nitrogenous constituents in hot water extracts of the dressed tissue of snapping turtles which were cultured with different diets, and reported that the amounts of free amino acids, nucleotides, creatine, and creatinine fluctuated among the specimens and that the main component in the nitrogenous compounds was gelatin, whose nitrogen accounted for 72-80% of total nitrogen in the hot water extracts.

Most probably, gelatin is derived from collagens in skin, tendon, and bone of snapping turtles, and is expected to play an important role in producing the taste of snapping turtle soup. This paper deals with the changes in content and molecular weight of gelatin in the hot water extracts of the dressed tissue of snapping turtle during heating.

Materials and Methods

Materials and Preparation of Hot Water Extract

Two specimens of snapping turtle Trionyx sinensis japonicus, cultured at Maisaka, Shizuoka Prefecture, were used. The turtles weighing about 800 g each were dressed in a usual manner, and the pieces dissected were wrapped with white cotton cloth in order to retain their shape during cooking, followed by heating with twice volumes of water at 90°C for 72 h.

Determination of Gelatin

The amount of gelatin was determined by the analysis of hydroxyproline in the acid-hydrolyzate of hot water extract according to the p-dimethylaminobenzaldehyde method.2)

SDS-Polyacrylamide Gel Disc Electrophoresis

A hot water extract prepared was mixed with an equal volume of 0.2 M phosphate buffer, pH 7.0, containing 2% SDS and 50% glycerol. Electrophoresis was carried out on 5% polyacrylamide gels using a buffer of 0.1% SDS and 0.1 M phosphate, pH 7.0, at 8 mA per gel for about 4 h.3) Following electrophoresis, the gels were stained with 10% Coomassie Brilliant Blue and destained with 10% acetic acid containing 10% isopropanol. Calf skin collagen (Nippi Co. Ltd.) was used as a standard sample.

Gel Filtration

Hot water extract was mixed with an equal volume of 0.1 M phosphate buffer, pH 7.0, containing 0.1 M sodium chloride, and the mixture was warmed up to 40°C and applied to a 1.6×100 cm column of Sephadex G-200. Elution was achieved by 0.05 M phosphate buffer, pH 7.0, containing 0.05 M sodium chloride at 40°C. The amount of gelatin in the eluate was determined from hydroxyproline content of acid-hydrolyzate. The Sephadex column was previously calibrated with calf skin collagen and gelatin (Nippi Co. Ltd.). The molecular weight of gelatin in the hot water extract was estimated from the calibration curve, a semilogarithmic plot of the molecular weight versus Kav: where Kav=(Ve−Vo)/(Vt−Vo); Ve, Vt, and Vo represent elution volume, total
volume of the gel bed, and void volume, respectively.

Results and Discussion

Changes in Total Nitrogen and Gelatin Nitrogen

Changes in the total nitrogen and gelatin nitrogen in the hot water extract of the dressed tissue of snapping turtle during heating are shown in Fig. 1. It can be seen that the amount of gelatin nitrogen increased rapidly during the initial heating period and reached as high as 1,010 mg (mg per 100 g of turtle, the same applying hereinafter) after 24 h. Total nitrogen level rose, in a similar manner, to 1,400 mg finally and the gelatin nitrogen accounted for 67-79% of the total nitrogen throughout the heating period. When compared with the result in the previous study,\(^1\) the increase of both total nitrogen and gelatin nitrogen was somewhat gradual but these final values were higher. This discrepancy might arise from the individual difference of the snapping turtle used and the heating condition adopted, because the rate of conversion of collagen to gelatin is known to be affected by the pH of the solution, the size of pieces, the temperature reached, and the denseness and kind of collagen.\(^4\)

Sugita et al.\(^5\) examined the change of pig skin collagen to gelatin by heating in water, and found that the amount of gelatin solubilized during heating increased with the lapse of time and reached a maximal value depending upon the temperature. Hughes\(^6\) studied the changes in content of extractive nitrogen of herring meat during heat processing at 116°C and found that the increase in levels of extractive nitrogen was mainly due to the conversion of connective tissue collagen to gelatin. It is obvious from their and our findings that collagen in animal tissues is solubilized by heating and gelatin thus formed increased rapidly up to the maximal level.

Degradation of Gelatin

Fig. 2 shows electrophoretic patterns of gelatins in the hot water extracts collected after heating for 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h. As indicated in the left column, the calf skin collagen used as a standard shows \(\alpha\), \(\beta\), and \(\gamma\) components whose molecular weights are about 100,000, 200,000, and 300,000, respectively. In the gelatins of snapping turtle heated for 0.5, 1, and 2 h, there were some-
what faint bands corresponding to $\alpha$, $\beta$, and $\gamma$ components, but these bands disappeared in the columns for the sample heated for more than 4 h. This result indicated that gelatins obtained after the short time heating still contained the main components of collagen. Except for these, clear bands did not appear in all samples, and a broad area was stained in all columns. Similar results were obtained when calf skin gelatin was submitted to SDS-polyacrylamide gel electrophoresis. Although the estimation of molecular weight of gelatin by the electrophoresis was difficult, gelatins formed after 4–12 h seemed to be widest in the distribution of molecular weight. When heated for a long time, the stained area shifted toward the bottom side, and it was suggested that gelatin broke down into degradation products having a smaller molecular weight.

Shirai et al.\textsuperscript{7} examined the gelatinization of pig skin collagen during heating, and reported that a narrow distribution of molecular weight could be produced under a certain condition. Sugita et al.\textsuperscript{5} reported an increase in degradation products of gelatin during heating of pig skin collagen. From these results, prolonged heating could break out not only inter- and intra-molecular crosslinks of insoluble collagen but also peptide bonds of the collagen back bone.

**Change in Molecular Weight of Gelatin**

The gelatins in the hot water extracts were chromatographed by a gel filtration. As depicted in Fig. 3, gelatins eluted as broad peaks, which meant that gelatins and their degradation products dispersed widely. Two peaks were observed in the chromatograms within 8 h, and the peaks gathered and shifted toward the right side as the heating time lengthened. These results also indicate the degradation of gelatin during heating. Molecular weights of gelatins in the main peaks were then estimated on the basis of their elution position from the calibrated Sephadex column and calculated to be 200,000 and 100,000 after 1 h, 100,000 and 50,000 after 4 h, 50,000 and 35,000 after 8 h, 28,000 after 12 h, 8,000 after 24 h, 4,000 after 48 h, and 3,000 after 72 h. These results seemed to agree with the view that the degradation of gelatin could occur at 80°C as far as the molecular weight reached about 10,000, and it was very difficult to split the peptide links of particles of about 4,000 molecular weight.\textsuperscript{8}

Usually, soup stock of snapping turtle is prepared by cooking for about 1.5 h,\textsuperscript{9} and the stock is thought to contain gelatin with a molecular weight of around 100,000. In our preliminary experiment, when a small amount of degradation product prepared by the enzymatic hydrolysis of gelatin was added to a synthetic amino acid mix-

![Fig. 3. Sephadex G-200 gel chromatograms of the hot water extracts of snapping turtle.](image-url)
ture, the taste was improved in mildness and viscosity. Therefore, the conversion of gelatin to lower molecular weight compounds during the cooking of snapping turtle soup seemed to expect the potential effects on the taste characteristic of the soup.

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References