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CHANGE OF POLYPEPTIDES IN BEEF SOUP STOCK DURING SIMMERING

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Background

Beef soup stock is the seasoned liquid in which beef meat has been simmered for hours, and is often used as main foundation for many kinds of soups and sauces. It has a rich color, robust flavor, and good body. It is recognized that 5'-inosin monophosphate (IMP) is a main component of umami taste which makes soup stocks delicious. In the preparation of beef soup stock, however, IMP was almost completely extracted from beef meat within 30 minutes' simmering (Tajima et al., 1991). This indicates that more prolonged simmering is required to extract other components of soup stock. The dissolution of stroma protein into soup stock is generally recognized to be the result of the gelatinization of collagen. Migita (1969) has reported that the gelatinization of collagen improves the taste of fish soup stock. Kim et al. (1994) have shown that fish skin gelatin hydrolysates have a brothy and sweet taste. However, exractability of collagen or gelatin from beef into beef soup stock as polypeptide constituents has not been elucidated so far.

Our objective of this study was to investigate the solubilization of collagen from beef meat into its soup stock as polypeptide constituents during simmering .

Methods

Cooking procedures. Beef round meat stored at 1℃ for 3days after slaughter was obtained from a commercial source and was stored at 0℃ for 17days, followed by storage at –35℃ until use. Meat was thawed at 5℃ for one night and cut into 30g cube before cooking. After 30g of beef cube had been soaked in 100ml of distilled water for 20 min(unheated extract), it was simmered at 95℃ for 0.5, 1, 2, 3, 6, and 15h, respectively. During simmering, the evaporated water was replenished with the distilled water previously heated to 95℃. The resulting extract was filtered through Toyo No.5 filter paper and made up to 100ml with distilled water.

Protein concentration. Protein concentration was measured by Biuret method using bovine serum albumin as a standard.

Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Tricine-PAGE). Tricine-PAGE was performed according to the method of Schagger et al. (1987). Forty μg of each protein sample was applied onto the gel. PAGE was carried out on the gel using SDS-Tricine discontinuous buffer system. Stacking gel contained 4%T and 3%C acrylamide, space gel 10%T and 3%C, and separating gel 16.5%T and 3%C, respectively. Approximate molecular weights were determined using broad range (Bio-Rad) and low molecular weight standards (Promega).

Western blotting. The polypeptides separated by Tricine-PAGE were electophoretically (5 hr at 60V) transferred from the gel to a nitrocellulose membrane (0.45 μm, Bio-Rad) in a Trans-Blot cell unit (Model 250/2.5, Bio-Rad) using 25mM Tris, 192mM glycine, and 10% (v/v) methanol buffer (pH 8.39). After the blocking, the membrane was incubated in type I-collagen polyclonal antibody solution at 2℃ for 16 h. The following treatment was made according to a usual precedure (Nakaya, 1994).

Reversed phase high performance liquid chromatography (RP-HPLC). RP-HPLC was performed on the extracted polypeptides in beef soup stock using a Vidac C18 column (250x4.6 mm). Elution was made by applying a gradient to the column using solvent A (0.06% trifluoroacetic acid (TFA) in H_2O) and solvent B (0.052% TFA and 80% acetonitrile in H_2O) as follows: 100% A for 5 min, 0 – 30% B at a rate of 1.2%/min. Then, the concentration of B increased stepwise as follows: 30% B for 10 min, 33% for 15min, 40% B for 20 min, 50% for 15min and 100% for 15 min. Flow rate was 0.5 ml/min and detection was at 210 nm.

Results and Discussions

The effect of simmerng time on the extraction of polypeptides from beef cubes into soup stock was investigated (Fig.1). Simmering of beef cubes at 95℃ rapidly increased the concentration of extractable polypeptides from beef cubes into beef soup stock during first 30 min and then it increased slowly for the following 3 hour and then it increased again with increasing simmering time. Fig.2 showed ttricine-PAGE patterns (A) of unheated extract and those of the extracts obtained after the simmering for 0.5, 1, 2, 3, 6, and 15 h, respectively, and was then subjected to western blotting(B) using type I-collagen antibody. Several bands having molecular weights between 97 to 17.2kDa were observed in the unheated extract(lane 1). This result is good agreement with PAGE pattern of soluble fraction of beef extracted into 20mM HEPES buffer, pH 7.2, at 20℃reported by Spanier et al(1990). In the beef soup stock obtained after simmering for 3 h, most of their bands(97-17.2kDa) gradually disappeared, while some of the bands having molecular weights of 42, 39, 35, 25, and 17.2 kDa were observed, but the intensity of those bands decreased. The western blotting patterns (B) using type I-collagen antibody revealed that bands having molecular weights between 200 to 30 kDa were originated from collagen and that the intensity of those bands increased after 3 h's simmering. Furthermore, additional bands of 22 and 18 kDa were observed in the blots of 6 and 15 h simmered soup stock.

The RP-HPLC elution profile of the extracted polypeptides from beef cubes simmered for 3 h is shown in Fig.3. The extracted polypeptides were separated into 4 fractions (Fr 1 to 4 in Fig.3). Time course of the change of the peak area of those four fractions is shown in Fig.4. Fractions 1 and 2 (Fr.1 and Fr.2 in Fig.4) were remarkably increased with increasing the simmering time and the

fraction 2 in the soup stock obtained after 3 h simmering was reacted with type I-collagen antibody. More than 3 h's simmering is usually required to obtain a good quality soup containing sufficient amount of collagen in it.

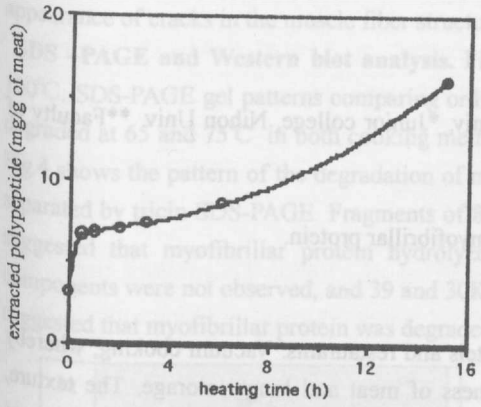

Fig. 1 The effect of simmering time on the extractable polypeptide content in beef soup stock

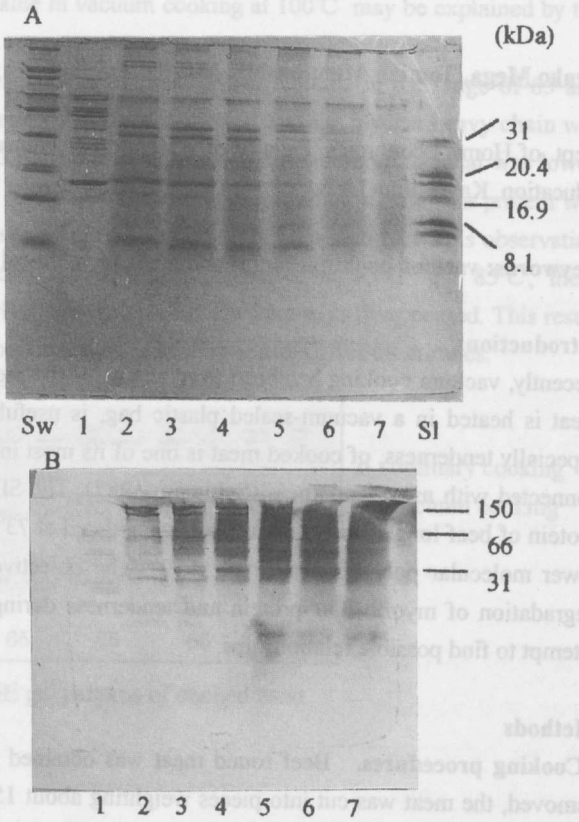

Fig.2 Tricine-PAGE pattern (A) and western blotting pattern (B) detected with type I-collagen antibody of unheated beef extract and beef soup stock

Lane Sw: wide range standard, lane 1: unheated beef extract, lane2: 0.5h simmering, lane 3: 1h simmering, lane 4: 2h simmering, lane 5: 3h simmering, lane 6: 6h simmering, lane 7: 15h simmering, Sl: low range standard

Conclusions

Extraction of collagen derivatives from beef into its soup stock was investigated. The Western blot with collagen antibody and fractions from RP-HPLC showed that collagen derivatives in beef soup stock increased with increasing simmering time of beef meat.

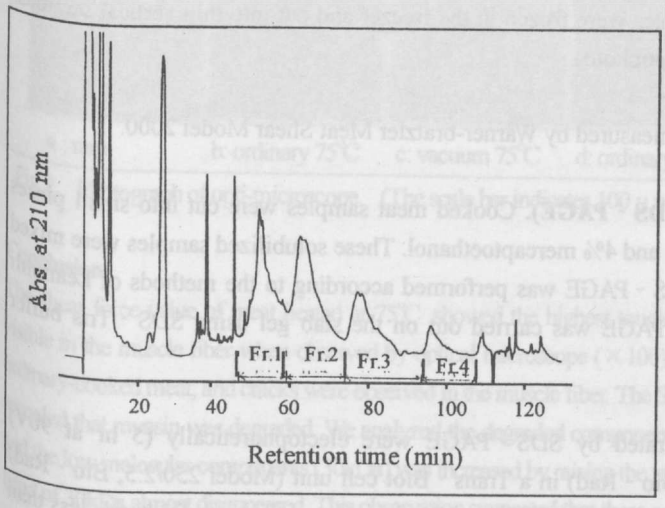

Fig.3 Chromatogram of the polypeptides in soup stock extracted from beef cubes after simmering for 3 h

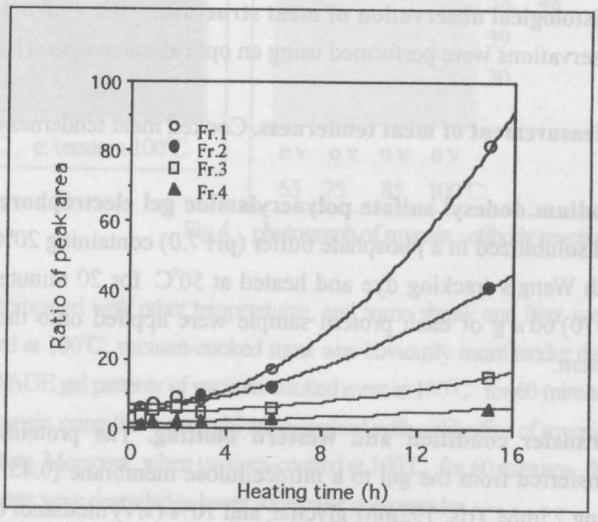

Fig.4 Time course of the change of the peak areas of four fractions (Fr.1 - Fr.4) in the chromatogram shown in Fig.3

Area of each fraction was calculated as the ratio to that of the fraction 4 of the soup stock obtained after 3 h simmering

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