Heat-Induced Effect on Soluble Proteins in Meat Soup Stock

Mariko Tajima, Tomiko Mitsuhashi, Ayako Mega* and Nobuhiko Arakawa**

Faculty of Education, Kagoshima University, Kagoshima 890, Japan
* Nihon University, Junior College, Mishima, Shizuoka 411, Japan
** Faculty of Education, Yamanashi University, Kofu 400, Japan
*** Faculty of Home Economics, Ochanomizu University, Bunkyo-ku, Tokyo 112, Japan

The heat-induced gelatinization of collagen and the solubilization of myofibrillar proteins in meat during the preparation of soup stock were investigated. The ratio of gelatin originating from collagen to the total soluble protein in soup stock was about 30% after heating for 6 hr. When the purified myofibrillar proteins were heated, most disappeared from the SDS-polyacrylamide gel electrophoretic pattern, and 40,000-, 23,000- and 10,000-18,000-dalton proteins appeared. The molecular weights of these proteins were similar to those of the proteins in soup stock. Furthermore, the content of heat-soluble protein from myofibrils increased gradually with increasing heating time.

The content of soluble proteins was lower when a mixture of sarcoplasmic and myofibrillar proteins was heated than when they were heated individually. A mixture with a higher ratio of sarcoplasmic proteins to myofibrillar proteins gave a more intense protein band of 10,000-18,000 daltons by SDS-polyacrylamide gel electrophoresis, the content of this low molecular weight protein increasing with increasing heating time.

These results suggest that some proteins in soup stock originate from not only stroma proteins, but also from sarcoplasmic and myofibrillar proteins.

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INTRODUCTION

In our previous papers, it was reported that the sarcoplasmic proteins in meat were dissolved in water during soaking, and that 90% of these dissolved proteins passed into “aku,” which is a complex of coagulated proteins, fat and minerals, during heating. There subsequently remained three main proteins of 40,000, 23,000 and 18,000 daltons in the soup stock. It was also suggested that the 40,000- and 23,000-dalton proteins originated from the non-sarcoplasmic fraction which contained stroma and myofibrillar proteins, and that the 10,000-dalton protein originated from both sarcoplasmic and non-sarcoplasmic proteins. The dissolution of stroma protein into soup stock is generally recognized to be the result of the gelatinization of collagen, and Migita has reported that the gelatinization of collagen improved the taste of soup stock.

On the other hand, the dissolution of proteins or polypeptides from myofibrils has not previously been investigated, because myofibrillar proteins are commonly recognized to form aggregates by heating. However, our preliminary study suggested the possibility of solubilization of some myofibrillar proteins which might affect the taste of soup stock, the muscle conformation, and the tenderness of meat.

The present experiment was conducted to investigate the gelatinization of collagen and to confirm the degradation of myofibrillar proteins in beef meat.
METHODS

Preparation of soup stock

Fresh beef round meat was obtained from a retail meat shop. After 30 g of the meat had been soaked in 100 ml of distilled water for 20 min, it was boiled for 0.5, 1, 2 or 3 hr. During boiling, the evaporated water was replenished with boiling distilled water. The soup stock thus obtained was filtered through Toyo No. 2 filter paper and made up to 100 ml with distilled water.

Measurement of proteins and hydroxyproline

The protein concentration was measured by the buret method.4 The gelatin from collagen dissolved in the soup stock was estimated from the hydroxyproline content measured by the p-dimethylaminobenzaldehyde method.5 The soup stock sample was hydrolyzed with 6 N HCl at 110°C for 16 hr, and the liberated hydroxyproline was determined. The conversion coefficient of hydroxyproline to collagen was calculated to be 8.72 on the basis of the amino acid composition of beef collagen.

Isolation of myofibrillar and sarcoplasmic proteins

The purified myofibrils were prepared from fresh beef round meat by using the procedure of Stromer and Goll.6 Sarcoplasmic proteins were prepared by homogenizing the ground beef round meat in 2 volumes of distilled water with a Waring blender. The homogenate was centrifuged at 1,500 × g for 15 min to give a supernatant containing sarcoplasmic proteins.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Soluble proteins in the soup stock and in the heated sarcoplasmic and/or myofibrillar proteins were analyzed by SDS-PAGE, which was conducted by the method of Weber and Osborn7 with some modifications as previously described.2 The final concentrations of the protein samples for SDS-PAGE analysis were 10% (v/v) 2-mercaptoethanol, 1.7% (w/v) SDS in 50 mM sodium phosphate buffer (pH 7.1), 6.7% (v/v) glycerin and 0.01% (w/v) marakaitigreen. The samples were heated at 100°C for 10 min, and scanning of the stained gels was carried out with a densitometer (Asuka Kogyo, OZ802).

RESULTS AND DISCUSSION

Dissolution of gelatin from collagen to soup stock

The ratio of collagen to total solubilized proteins in the soup stock is shown in Fig. 1. The total protein concentration in the soup stock heated for 0.5 hr was the lowest and increased gradually as the heating time was prolonged. As the collagen concentration in the soup stock also increased with increasing boiling time, its ratio to total protein increased, although the ratio was less than 30% after 6 hr of boiling. This suggests the possibility that the myofibrillar proteins were solubilized into soup stock during boiling. Kiguchi and Kobayashi8 have reported that collagen produced a smaller protein of 18,000 daltons by heating at 80°C for 20 min, so that the protein in soup stock with a similar molecular weight may have originated from collagen. Therefore, studies are now underway to confirm whether the 10,000–18,000-dalton proteins are actually derived from collagen.

Heating of myofibrillar proteins

We described in the previous paper2 that the 40,000-, 23,000- and 10,000-dalton proteins seem to have originated from the non-sarcoplasmic fraction, although the ratio of the derivatives from collagen to the total proteins in soup stock was not high enough to confirm those three proteins as being derivatives from collagen. Hence, we investigated the formation of soluble derivatives from myofibrillar proteins by boiling.

Purified myofibrils suspended in 0.15 M KCl...
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were heated in a boiling water bath for various lengths of time. The suspension was then centrifuged at 1,500 × g for 15 min to separate the supernatant containing the heat-soluble derivatives, and this supernatant was subjected to SDS-PAGE. Figure 2 shows the SDS-PAGE patterns of the heat-soluble derivatives from myofibrils. At 0 hr, the myofibrils had myosin, actin, α-actinin and some other proteins. However, most of these had disappeared after heating for 30 min, and bands of 40,000, 23,000 and 18,000 daltons appeared instead. As the heating time was prolonged, an additional peak of 70,000 daltons appeared, while peaks of 10,000–18,000 daltons could still be observed. Most of these peaks coincide with those found in the SDS-PAGE patterns of the proteins in soup stock. This result demonstrates that some of the proteins solubilized from meat into soup stock seem to have originated from myofibrils. However, since purified myofibrils were heated in this study, the heating conditions such as pH and ionic strength were different from those used for preparing soup stock from whole meat.

The solubilized protein content of the supernatant separated from heated myofibrils is illustrated in Fig. 3, demonstrating that it increased gradually with increasing heating time. This suggests that heating might also have induced partial degradation of the myofibrillar proteins, which may eventually have resulted in solubilization.

**Effect of the coexistence of sarcoplasmic and myofibrillar proteins on solubilization by heating**

Our preliminary experiments demonstrated that 10% of sarcoplasmic proteins remained in the supernatant after heating at 100°C for 3 hr. The present study also shows that some soluble derivatives were produced from myofibrils by heating. Since meat contains both sarcoplasmic and myofibrillar proteins, the effect of their coexistence on solubilization by heating was investigated. The total concentration of sarcoplasmic and myofibrillar proteins was fixed at 7 mg/ml, and their ratio was varied as shown in Fig. 4. The mixtures were adjusted to 0.15 M KCl and heated in a boiling water bath for 30 min or 3 hr. They were then centrifuged to remove the insoluble materials, and the protein content of each supernatant was determined. The results are shown in Fig. 4, the protein content of the supernatant with the 10:0 ratio being higher than that with the 0:10 ratio. This suggests that more soluble proteins were produced by heating from sarcoplasmic proteins than from myofibrillar proteins. In case of the myofibrillar proteins, longer heating produced more soluble derivatives, while this did not occur in the case of the sarcoplasmic proteins, as was observed in our
When both proteins were mixed, the content of soluble derivatives from the mixture with 2:8, 4:6, 6:4 and 8:2 ratios was lower than each theoretical value on a straight line constructed between the two points for the 10:0 and 0:10 ratios. This demonstrates that the coexistence of these proteins decreased the solubility of the derivatives by heating. Because myofibrils have a fibrous structure and are susceptible to denaturation by heating, they may bind the sarcoplasmic proteins when coagulating and the aggregate may become more complex. Thus, their coexistence may have an effect on the clarity of soup stock.

The SDS-PAGE patterns of soluble derivatives from a heated mixture of sarcoplasmic (SP) and myofibrillar (MF) proteins are shown in Fig. 5. The SDS-PAGE pattern for 30 min of heating shows 40,000-, 23,000- and 18,000-dalton proteins in the mixtures with a higher ratio of myofibrillar proteins. On the other hand, in the mixtures containing more sarcoplasmic proteins, the 10,000-18,000-dalton peaks are higher. The SDS-PAGE patterns after 3 hr of heating demonstrate that the lower molecular weight proteins were increased by prolonged heating. These results suggest that 40,000- and 23,000-dalton proteins originated from myofibrils, while 10,000–18,000-dalton proteins originated from myofibrils and sarcoplasm.

In summary, the present research demonstrates that, when meat was heated with water at 100°C, collagen in the connective tissue was degraded to gelatin. Furthermore, part of the myofibrillar proteins was degraded into 40,000-, 23,000- and 18,000-dalton derivatives, and part of the sarcoplasmic proteins into 10,000–18,000-dalton derivatives, all of which were similar in molecular weight to the proteins found in soup stock.

REFERENCES
肉スープストック中の可溶性タンパク質の由来について

田島真理子，三橋富子*，妻鹿緑子**，荒川信彦***

（鹿児島大学教育学部，* 日本大学短期大学部，** 山梨大学教育学部，*** お茶の水女子大学家政学部）

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スープストック調製時に熱によっておこる肉中のコラーゲンのゼラチン化と筋原纖維タンパク質の可溶化について検討した。スープストック中の全可溶性タンパク質量に対するコラーゲン由来のゼラチンの割合は6時間加熱で約30％であった。熟製した筋原纖維を加熱すると、ほとんどのタンパク質は変性して SDS-ポリアクリルアミドゲル電気泳動パターンから消失し、加熱可溶性画分には40,000, 23,000, 10,000~18,000ダルトンのタンパク質のみが認められた。これらのタンパク質の分子量はスープストック中のタンパク質の分子量に近似していた。また、筋原纖維タンパク質を加熱した後の可溶性タンパク質量は加熱時間の増加に従って次第に増加した。

筋原タンパク質と筋原纖維タンパク質の混合物を加熱した場合、可溶性タンパク質量は、それぞれ加熱した場合より減少した。筋原纖維タンパク質に対する筋原タンパク質の割合が高い場合、加熱可溶性タンパク質の SDS-ポリアクリルアミドゲル電気泳動パターンでは10,000~18,000ダルトンのバンドがより強く現れた。また、加熱時間の増加に伴って低分子タンパク質が増加した。

これらの結果から、スープストック中には筋原タンパク質、筋原糸維タンパク質、結合組織タンパク質に由来するそれぞれのタンパク質が存在していると考えられる。

キーワード: 肉, 筋原糸維タンパク質, 筋原タンパク質, 加熱, ゼラチン化, スープストック.