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CHANGES IN PEPTIDES DURING CONDITIONING OF THREE TYPES OF BEEF SAMPLES

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Background:

Muscle proteolytic enzymes, calpains (I and II) and lysosomal proteases (cathepsins B and L), appear to be involved in the conditioning process of meat and these proteases may enhance each other's effects. Proteolysis of myofibrillar proteins appears to be a major contributor to tenderization during post-mortem storage. These proteases may also act on sarcoplasmic proteins, and, depending on the time of the conditioning, produce peptides and free amino acids. The amount of peptides and free amino acids are important factors in the conditioning of meat and they improve the flavor or taste of the meat. The number of reports on the effect of sarcoplasmic proteins is small when compared with that of myofibrillar proteins.

Objectives:

We conducted this study to gain knowledge about the peptides which proteins contributed to peptide content and what peptide components were produced.

Methods:

M. Semitendinosus muscles were obtained from 8 Holstein steers 2 days after slaughter and 3 types of beef samples were prepared: whole muscle, homogenate and sarcoplasma. The homogenate sample was prepared as follows: minced meat was added in a 1:2 ratio to the 30 mM citrate phosphate buffer containing 0.05% NaN_3 and 0.1 M NaCl, pH 5.5. The sarcoplasma was supernatant obtained from a part of the homogenate sample by centrifugation (40,900 x g, 30 min, 0 ℃) and then filtered by a 0.25 μ m pore sized filter. The 3 types of beef samples were stored at 4 ℃ until 28 days after slaughter.

The other samples were prepared in much the same way. The whole muscle and homogenate samples were homogenized and centrifuged to get the supernatant. Each supernatant obtained from the beef samples was heated at 75℃ for 15 min. or mixed with an equal volume of 4% TCA to remove the proteins. These 2 types of peptide fraction were named the heat soluble peptides and the 2% TCA soluble peptides.

Peptide content was determined by the Lowry method. SDS-PAGE was performed using a 16.5% Polyacrylamide gel with a 6% cross link containing Tricine (0.1 M) in the running buffer.

Size exclusion chromatography was performed using a Superdex peptide HR 10/30 column (Amersham pharmacia).

Results and Discussion:

Contents of the heat soluble peptides are shown in Fig. 1. Peptide content was 250.7 mg/100g beef 2 days after slaughter and was higher than that of the 2% TCA soluble peptides. During conditioning, peptide content increased in this order; homogenate, whole muscle and sarcoplasma, and they reached 574.3 mg, 519 mg and 497.8 mg 28 days after slaughter, respectively.

The peptide level for the 26th day was 268.3 mg in the whole muscle, 323.9 mg in the homogenate and 247.1 mg in the sarcoplasma. The whole muscle and homogenate contained myofibrillar proteins, but they were missing in the sarcoplasma. The difference in the amount of peptides between the whole muscle and sarcoplasma was 21.2 mg and 76.8 mg between the homogenate and sarcoplasma samples. As these numbers show the percentage of contribution by myofibrillar proteins was 7.9% in the whole muscle, and 23.7% in the homogenate. These facts indicate that the increment of change in peptide content during conditioning mainly originates from the sarcoplasmic proteins.

Content of the 2% TCA soluble peptides was 157.4 mg 2 days after slaughter and it was about 90 mg lower than

that of the heat soluble peptides. This difference between the heat soluble and 2% TCA soluble did not change in the 3 samples during conditioning.

The value of 157.4 mg of the 2% TCA soluble peptides was similar to the previous data [1]. The values 28 days after slaughter were 412.7 mg, 486.4 mg and 390.3 mg in the whole muscle, homogenate and sarcoplasma, respectively. These values, 486.4 mg and 390.3 mg, were higher than that of previous data [1]. This may be due to the difference between Holstein steers and Hereford steers. The peptides from myofibrillar proteins were 22.4 mg and 96.1 mg in the whole muscle and homogenate, respectively. The percentages from myofibrillar proteins were 8.8% and 29.2% for increment peptides 28 days after slaughter.

SDS-PAGE pattern of the heat soluble peptides from the whole muscle is shown in Fig. 2. The main component was 17 kDa and this was assumed to be myoglobin. Others were 12.2 and 8.0 kDa components. During conditioning, 42, 30, 23, 14, 10.7, 7.0 and 5.5 kDa components increased gradually.

Fig. 3 shows the heat soluble peptides from the sarcoplasma. The peptide components were mostly low molecular components below 17 kDa except one which was 23 kDa. Increment components were 10.7, 8.0 and 5.5 kDa and main component was 8 kDa. The difference between the whole muscle (Fig. 2) and sarcoplasma (Fig. 3) was if they contained myofibrillar proteins or not. It is thought that high molecular peptides, (42, 30 and 23 kDa components, Fig. 2), originated from myofibrillar proteins.

Conclusion:

The peptides that increased during conditioning mainly originated from sarcoplasmic proteins, and the contribution ratio to produce peptides was between 76.3 - 92.1% in the heat soluble peptides. The peptides from myofibrillar proteins were high molecular peptides, with 42, 30 and 23 kDa components and the peptides from sarcoplasmic proteins were low molecular; with mainly 8.0 and 5.5 kDa components.

Literature:

1) Mikami, M., M. Nagao and M. Sekikawa, H. Miura and Y. Hongo. Anim. Sci. Tech.(Jpn), 69: 53-61, 1998.

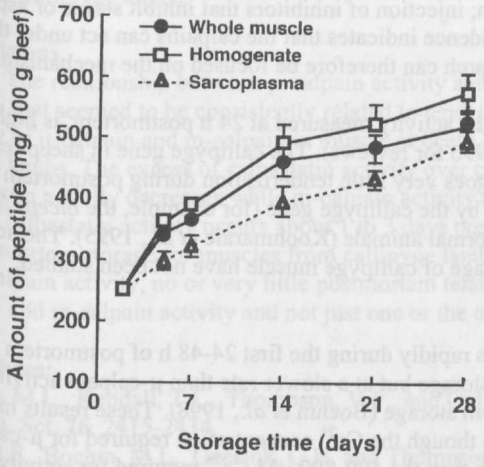

Fig. 1. Content of the heat soluble peptides in 3 types of beef samples.

Bovine Semitendinosus muscles were obtained from Holstein steer. Three types of beef samples were whole muscle, homogenate and sarcoplasma, and stored at 4°C until 28 days after slaughter. Each supernatant obtained from 3 types of beef samples was heated at 75°C for 15 min.

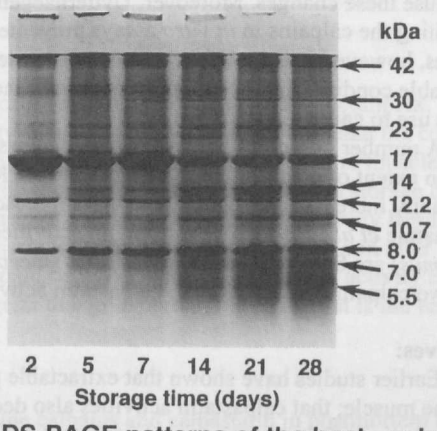

Fig. 2. SDS-PAGE patterns of the heat soluble peptides from the whole muscle.

Polyacrylamide gel was 16.5% and cross link was 6%. Samples were separated with a running buffer containing 0.1 M Tricine.

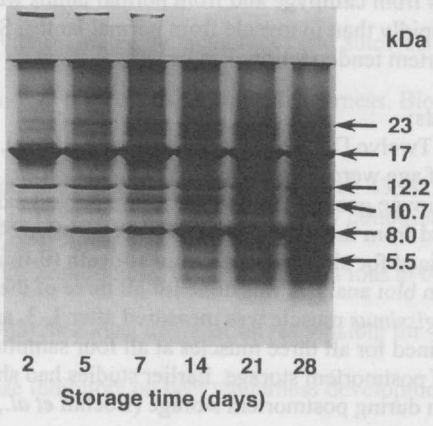

Fig. 3. SDS-PAGE patterns of the heat soluble peptides from the sarcoplasma.