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PEPTIDE AND FREE AMINO ACID CONTENT OF ELECTRICALLY STIMULATED BEEF

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Please refer to Folio 17A.

INTRODUCTION

Many studies have been carried out on the effects of ES during post-mortem storage. Muscle proteolytic enzymes, calpain (CANP) and lysosomal proteases (cathepsins B and L), appear to be involved in the conditioning process of meat, and there may be a cooperative mechanism between these different proteases (Penny and Ferguson-Pryce, 1978). Dutson *et al.*(1980) have also reported that lysosomal membranes are disrupted due to ES and that the activity of free lysosomal enzymes increases. Therefore, the possibility for increased tenderness of ES meat could be due to the increased rate of enzymes release from the lysosomes into the rapidly acidifying environment within the muscle fibre and the increased action of these enzymes at low pH.

Proteolysis of myofibrillar proteins appears to be a major contributor to the tenderization process during post-mortem storage (Etherington, 1984; Pearson *et al.*, 1983). It is also thought that these proteases act on sarcoplasmic proteins to produce peptides and amino acids. However, there are few data on the effect of ES on sarcoplasmic proteins. In our preliminary experiment, it was found from HPLC patterns that the sarcoplasmic protein content decreased, and the peptide and amino acid content increased in ES muscle (Mikami *et al.*, 1991).

The objective of this experiments was to investigate the proteolysis of sarcoplasma and the accumulation of peptides and free amino acids in the stored muscle homogenate and the stored sarcoplasma.

MATERIALS AND METHODS

Preparation of ES muscle homogenate and sarcoplasma

Nine Hereford steers, 25 months old and 650kg average live weight, were slaughtered. ES of low voltage (40V, 13.8Hz) was carried out on each of three steers for 30 or 60 seconds within five minutes after slaughter. Three steers were used as controls. Two hours after slaughter samples of *biceps femoris* muscles were obtained and homogenized three times with 30mM citrate-phosphate buffer containing 0.1M NaCl and 0.05%NaN_3 (pH5.5) which was three times the volume of the muscle sample. The pH of these homogenates was near 5.6 in each case.

The homogenate was divided into two portions with reagent added as follows: no additive; and 200μM leupeptin plus 5mM EDTA (LE). These homogenates were divided into two portions again and one part was stored at 1±1°C for 2, 7, 14 and 21 days after slaughter.

The other part of the homogenate was prepared for sarcoplasma as follows. It was centrifuged at 11000xg for 20 minutes at 1°C. The supernatant was filtered through Toyo No.5c filter paper. The two resulting kinds of sarcoplasma, one with no reagent and one with LE added, were also stored under the same conditions as the homogenate. These samples were prepared for peptide and free amino acid content analysis 0, 2, 7, 14 and 21 days after slaughter.

Determination of peptides and amino acids

Samples for analysis of peptides and amino acids were prepared from the stored homogenate and the stored sarcoplasma. After storage, 15g of the homogenate was centrifuged at 11000xg as described above. The supernatant (sarcoplasma) was mixed with an equal volume of 4% TCA solution, incubated at 37°C for 30 minutes and centrifuged at 4500xg for 10 minutes. In the case of the stored sarcoplasma, an equal volume of 4% TCA was mixed with it in the same way as with the homogenate samples. The filtrate was used for determination of peptides and amino acids (Figure 1). Peptide analysis was carried out using the Lowry method with bovine serum albumin as a standard. Free amino acid analysis was carried out on a fully automated amino acid analyzer (JASCO Model 8000 series) and was determined by the OPA reagent method.

RESULTS AND DISCUSSION

Peptide content

The results obtained for average peptide content are shown in Table 1. On the 0 day (six hours after slaughter) peptide content was significantly different among the control (162.5mg per 100g meat) and the ES30 and ES60 homogenates (192.1 and 198.6mg per 100g meat). These values increased at each day during storage and there was a significant difference among the control and the ES homogenates. Peptide content at 21 days came to 356.4, 409.7 and 404.5mg per 100g meat in the control, the ES30 and ES60 homogenates respectively. These increments of peptides in the ES30 and ES60 homogenates were 15.0 and 13.5% higher than those in the control. It is thought that in this experiment the high peptide content may be due to the high activity of proteases in the ES homogenates.

To investigate the origins of these peptides, whether they derived from myofibrillar proteins or sarcoplasmic proteins, peptide content was determined for stored sarcoplasma. In the case of the stored sarcoplasma, similar increasing tendencies were seen among the control and the ES homogenates during storage. However, the increments in the both the control and ES sarcoplasma were less than in the case of the stored homogenates. The increments in the control and the ES60 sarcoplasma were 71.2 and 73.7% of the homogenate at 21 days, respectively, but there were significant differences among the control and the ES sarcoplasma.

In a previous paper (Mikami *et al.*, 1991), the authors reported the peptide content for Holstein cows and at that time peptide content in the ES muscle was larger than that of the control. These facts indicate that ES causes the proteases to activate and to produce peptides from the proteins. Besides, they showed that the origins of these peptides were not only myofibrillar proteins but sarcoplasmic proteins also and that the ratio of production of peptides from the sarcoplasmic proteins was larger than that from myofibrillar proteins. George *et al.* (1980) reported that the precipitation of sarcoplasmic proteins was observed on the myofibrils of ES muscle via electron microscopy. Precipitation of sarcoplasmic proteins was also reported by Scopes (1964) and in this experiment it was also observed during storage. However, peptide content in the ES sarcoplasma was larger than that in the control.

Free amino acid content

Table 2 shows the total amount of free amino acids in the homogenate and the sarcoplasma stored at 1°C until 21 days after slaughter. Total amount at each day increased in both the control and ES homogenates during storage. However, there was no significant difference among the control and the ES homogenates at each day. The total amount also increased in the stored sarcoplasma, although it contained less than did the stored homogenate. The increments of free amino acids in the control, the ES30 and ES60 sarcoplasma were 87.5, 85.4 and 81.3 % of the homogenates at 21 days respectively. These facts indicated that increased free amino acids originated mainly in the peptides or sarcoplasmic proteins in the sarcoplasma rather than in the myofibrillar proteins. Although ES treatment caused peptides to be generated from both the homogenate and sarcoplasma (Table 1), it was not effective in increasing the total amount of free amino acids in the control and ES homogenates.

Remarkable change in each free amino acid was observed. In the case of glutamic acid, among the control and the ES homogenates (Table 3), the content was increased by ES treatment from 0 day, measuring 18.4, 21.4 and 25.2mg per 100g meat in the control, the ES30 and ES60 homogenates respectively. This increasing tendency was seen until 21 days after slaughter and the glutamic acid content in the control, the ES30 and ES60 homogenates at 21 days reached 23.3, 31.1 and 32.0mg per 100g meat respectively. Another amino acid increasing due to ES treatment was phenylalanine. On the other hand, decreasing amino acids were seen in the case of proline, tyrosine and arginine in the stored homogenates at 21 days.

In the stored sarcoplasma, glutamic acid content in the control increased rapidly and became the highest in each case at 21 days, when it was twice as much as in the control at 0 day, but the contents of the ES samples were not different from those of the stored homogenates. However, aspartic acid, alanine and methionine increased with ES treatment. In the case of the control sarcoplasma, the only difference in stored condition was the removal of myofibrils and there was no stimulation to proteases by ES treatment. From these facts, it is thought that myofibrils contain inhibitors of aminopeptidases and by removing the myofibrils, aminopeptidases probably work to breaks down substrates easily. However, the relation to ES treatment is not clear, but it is clear said that ES treatment changes the balance among the aminopeptidases, inhibitors and substrates.

Cathepsins and calpain are endogenous endopeptidases and generate peptides during the storage of beef. A high peptide content ensures adequate substrate for aminopeptidases and a high amino acid content in ES muscle may be due to aminopeptidases, such as aminopeptidase C and hydrolase (Nishimura *et al.*, 1988). In the homogenate containing 200μM leupeptin and 5mM EDTA, which are inhibitors of cathepsins and calpain, almost all free amino acids decreased, but their decrement was not large and total amounts of free amino acids at 21 days were 80.0, 82.0 and 86.9% of the normal homogenates in the control, ES30 and ES60 respectively. These facts indicate that the activity of cathepsins and calpain is not completely inhibited by 200μM leupeptin and 5mM EDTA, or that there may be other unknown proteases.

CONCLUSION

The conditioning of beef is thought to correspond to the storage of homogenates in this experiment. ES treatment causes the accumulation of peptides and glutamic acid, and it is thought that ES treatment contributes to the favourable taste of beef.

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Table 1. Changes in peptide content of homogenate and sarcoplasma during storage (mg/100g meat).

Days post-mortem	Homogenate storage Control	ES 30	ES 60
0*	162.5± 6.3	192.1± 0.9	198.6± 3.2
2	199.1± 2.7	229.1± 2.5	236.6± 1.3
7	270.7± 8.5	310.3± 5.5	307.3± 5.5
14	324.3±19.4	366.4± 6.8	367.4±12.0
21	356.4±26.4	409.7± 4.5	405.5±10.3

Days post-mortem	Sarcoplasma storage Control	ES 30	ES 60
0*	162.5± 6.3	192.1± 0.9	198.6± 3.2
2	182.5± 4.6	214.4± 3.2	214.6± 3.6
7	206.6± 6.7	242.9± 4.6	244.4± 2.0
14	226.1± 6.2	272.5± 6.3	270.5± 4.7
21	253.9± 9.1	291.8±10.9	299.0± 8.1

Beef homogenate was prepared from biceps femoris with 30mM citrate-phosphate buffer containing 0.1M NaCl and 0.05% NaNa (pH5.5) which was three times the volume of the muscle sample. Sarcoplasma was obtained from the homogenate by centrifugation and filtration (Toyo No.5c filter paper). Both homogenate and sarcoplasma were stored at 1±1 °C for 2, 7, 14 and 21 days after slaughter. Peptide content was determined by the method of Lowry. Data were expressed as an average of three cattle and SE.

* Zero days means six hours after slaughter.

Tables 2 and 3, and Figure 1 were not received. Please contact the authors for copies.