Effects of Electrical Stimulation on the Peptide and Free Amino Acid Contents of Beef Homogenate and Sarcoplasm during Storage

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Abstract To investigate the peptide and free amino acid contents of beef treated with electrical stimulation (ES), Hereford steers were slaughtered. ES was carried out at a low voltage (40V and 13.8Hz) for 0 (cont.), 30 sec (ES30) and 60 sec (ES60). Homogenate and sarcoplasm from *M. biceps femoris* muscles were prepared and stored at $2\pm 1^{\circ}\text{C}$ for 0 (6hrs), 2, 7, 14 and 21 days after slaughter. The peptide contents in the both ES30 and ES60 homogenates were greater than in the control from day 0 after slaughter. The amount of peptides in the control, the ES30 and the ES60 homogenates were 162.5, 192.1 and 198.6 mg per 100 g meat on day 0, respectively and 356.4, 409.7 and 405.5 mg per 100 g meat 21 days after slaughter, respectively. The total amount of free amino acids increased during storage, but did not significantly differ between the control and the ES homogenates. However, the content of Glu increased with ES treatment from day 0; measuring 23.3, 31.1 and 32.0 mg per 100 g meat in the control, the ES30 and the ES60 homogenates 21 days after slaughter, respectively. It is seemed that ES contributed to the improvement of meat flavor or taste, by increasing the peptides and free amino acids, especially Glu. In the stored sarcoplasm, the peptide and free amino acid contents were also investigated under the same conditions as the homogenate. Total amounts of peptides and free amino acids increased during storage in the sarcoplasm also, although these increment corresponded to 45.8-48.5% and 53.9-69.7% of the homogenate storage 21 days after slaughter, respectively. These findings indicate that origins of peptides and free amino acids are derived from myofibrillar proteins as well as sarcoplasm.

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Many studies have been carried out on the effects of ES during post-mortem storage; ES has been recognized as a means of improving tenderness of the muscle. This improvement is related to the avoidance of cold-shortening, increased muscle fibre fracture, accelerated ageing by lysosomal enzymes at low pH and to the effect of calpains in the very early post-

mortem period in which the pH is relatively high^{3,4,9,21)}. ES was also found to enhance the rate of troponin-T degradation during ageing of muscles^{1,10)}. Muscle proteolytic enzymes, calpains 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 effects¹⁸⁾. DUTSON *et al.*⁴⁾

have also reported that lysosomal membranes are disrupted and that the activity of free lysosomal enzymes increases after ES. Therefore, increased tenderness of ES meat may be due to the increased rate of enzymes released from the lysosomes into the rapidly acidifying environment within the muscle fiber and the increased action of these enzymes at low pH.

Proteolysis of myofibrillar proteins appears to be a major contributor to tenderization during post-mortem storage^{5,17}. These proteases may act on sarcoplasmic proteins to produce peptides and amino acids. However, there are not many reports^{14,15} on the effect of sarcoplasmic proteins compared with myofibrillar proteins. In preliminary experiments, HPLC patterns reveal that the sarcoplasmic protein content decreased, and the peptide and amino acid content increased in the ES muscle¹². The amount of peptides and free amino acids are important factors in the ageing of meat, and those improve flavor or taste of meat.

Many studies have reported about changes in myofibrillar proteins after ES^{1,3,19}, but there is little data on peptide and free amino acid content. The objective of this experiment is to examine the accumulation of peptides and free amino acids and the proteolysis of sarcoplasm in stored muscle homogenate and stored sarcoplasm treated with ES.

Materials and Methods

Preparation of ES muscle homogenate and sarcoplasm: Nine Hereford steers, 25 months old and 650 kg average live weight, were fed at Shintoku Animal Husbandry Experimental Station and slaughtered at Tokachi Meat Packer Inc. Low voltage (40V, 13.8Hz) ES was carried out on each of 3 steers for 30 or 60 sec within 5 min after slaughter. Three steers were used as controls. Two hours after slaughter, samples of *M. biceps femoris* were obtained and homogenized with 30 mM citrate-phosphate buffer containing 0.1 M NaCl and

0.05% NaN₃ (pH 5.5), which was three times the volume of the muscle sample. The pH of these homogenates was approximately 5.6 in each case.

The homogenate was divided into 2 portions, one of which was stored at $2 \pm 1^\circ\text{C}$ for 2, 7, 14 and 21 days after slaughter. The other portion was prepared for sarcoplasm as follows: It was centrifuged at $11,000 \times g$ for 20 min at 1°C . The supernatant was filtered through Toyo No. 5c filter paper. This sarcoplasm was 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 free amino acids: Samples for analysis of peptides and free amino acids were prepared from the stored homogenate and the stored sarcoplasm. After storage, 15 g of the homogenate was centrifuged at $11,000 \times g$ as described above. The supernatant (sarcoplasm) was mixed with an equal volume of 4% TCA solution, incubated at 37°C for 30 min and centrifuged at $4,500 \times g$ for 10 min. In the case of the stored sarcoplasm, an equal volume of 4% TCA was mixed with it in the same way as with the homogenate samples. The filtrate was used for analysis of peptides and free amino acids (Fig. 1). Peptide content was determined 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 average peptide contents of the samples are shown in Table 1. The effect of ES treatment on peptide production was observed soon after slaughter. On day 0 (6hrs) after slaughter, there was a significant difference in peptide content between the control (162.5 mg per 100 g meat), and the ES 30

and the ES60 homogenates (192.1 and 198.6 mg per 100 g meat, respectively). Similar results were reported on peptide contents for the ES homogenate of Holstein cows¹²⁾; which were also greater than the control 2 days after slaughter. These values increased during storage, and there were significant differences between the control and the ES homogenates on each day. Peptide content at 21 days grew to 356.4, 409.7 and 404.5 mg per 100 g meat in the control, the ES30 and the ES60 homogenates, respectively. The peptide contents in the ES30 and the ES60 homogenates were 53.3 mg and 49.1 mg higher than that of the control stored for 21 days after slaughter. The increasing amount of peptides in the ES homogenates was observed at all stages after slaughter as previously reported¹²⁾; peptide contents increased approximately two fold compared to six hours after slaughter. NISHIMURA *et al.*¹³⁾ reported that the peptide contents of beef rounds rose from 208 mg at 4 days to 251 mg per 100 g meat at 12 days; an insignificant change. However,

the peptide content in the control at 0 and 21 days in this experiment were similar to those of Japanese black cattle¹⁶⁾. From above values, one sees that ES treatment is effective in increasing peptide content. One may also conclude from this experiment that the high peptide content is due to the high activity of endogenous proteinases on the weakening of inhibitor for these in the ES homogenates.

DRANSFIELD²⁾ described the proteolysis mechanism that operates during meat tenderization when calpain I becomes active at about pH 6.3 or about 6 hrs after slaughter in beef. At about 16 hrs, calpain II becomes active. As ES hastens reduction in muscle pH, calpain I may play a role in post-mortem proteolysis in the ES meat at an early stage after slaughter, though only for a short period of high pH and high temperature.

To investigate the origins of these peptides, whether they derived from myofibrillar proteins or sarcoplasmic proteins, peptide contents were determined for the stored sarco-

Fig. 1. Experimental scheme and sample preparation.

Beef samples were obtained from *M. biceps femoris* of Hereford steers 2 hrs after slaughter.

Two % TCA solubles were analyzed for peptide and free amino acid contents.

plasma also. In the case of the stored sarcoplasm, similar increasing trend were also seen in the control and the ES homogenates during storage. However, the increments in the control and the ES sarcoplasm were less than that of the stored homogenates. Peptide contents in the control, the ES 30 and the ES 60 sarcoplasm were 253.9, 291.8 and 299.0 mg per 100 g meat at 21 days, respectively; there were significant differences between the control and the ES sarcoplasm. The increments of peptides were 91.1, 99.7 and 100.4 mg during storage for 21 days in the control, the ES 30 and ES 60 sarcoplasm, respectively. These values corresponded to 47.0, 45.8 and 48.5% of the values in the case of homogenate storage, respectively. This indicates that ES activates the endogenous proteinase which produces peptides from the proteins, and that these peptides ori-

ginate not only in myofibrillar proteins but also in sarcoplasmic proteins and that about half of the peptides produced during storage are derived from sarcoplasm.

GEORGE *et al.*⁶⁾ 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 SCOPES²⁰⁾ and in our experiment, precipitation was also observed during storage of sarcoplasm. However, peptide contents in the ES sarcoplasm were greater than in the control.

It is well known that there are two proteolytic systems of endogenous proteinases (calpains and cathepsins) in post-mortem muscle^{7,17,18)}. Recently, KOOHMAIE⁸⁾ reported the results of his experiments regarding the mechanism of meat tenderization during post-

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

Days post-mortem	Homogenate storage			Sarcoplasm storage		
	Control	ES 30	ES 60	Control	ES 30	ES 60
0*	162.5 ± 6.3	192.1 ± 0.9**	198.6 ± 3.2**	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**	182.5 ± 4.6	214.4 ± 3.2**	214.6 ± 3.6**
7	270.7 ± 8.5	310.3 ± 5.5**	307.3 ± 5.5**	206.6 ± 6.7	242.9 ± 4.6**	244.4 ± 2.0**
14	324.3 ± 19.4	366.4 ± 6.8*	367.4 ± 12.0	226.1 ± 6.2	272.5 ± 6.3**	270.5 ± 4.7**
21	356.4 ± 26.4	409.7 ± 4.5*	405.5 ± 10.3	253.9 ± 9.1	291.8 ± 10.9*	299.0 ± 8.1**

: Zero day refers to measurements 6 hrs after slaughter. Data are expressed as an average of 3 cattle and S.E. Asterisks (: 5%, **: 1%) show the significant level of mean differences between control and ES 30, and ES 60.

Table 2. Changes in total free amino acid content of homogenate and sarcoplasm during storage (mg per 100 g meat)

Days post-mortem	Homogenate storage			Sarcoplasm storage		
	Control	ES 30	ES 60	Control	ES 30	ES 60
0*	103.25 ± 2.34	104.11 ± 2.85	107.34 ± 6.93	103.25 ± 2.34	104.11 ± 2.85	107.34 ± 6.93
2	105.99 ± 1.73	112.77 ± 4.36	117.08 ± 9.73	105.54 ± 2.21	106.76 ± 4.25	116.53 ± 12.43
7	123.86 ± 2.67	122.65 ± 2.00	139.87 ± 12.37	120.97 ± 3.56	123.08 ± 6.92	130.35 ± 10.72
14	147.65 ± 6.46	152.88 ± 5.28	153.62 ± 9.53	129.58 ± 2.28	137.90 ± 4.82	132.57 ± 11.50
21	175.55 ± 5.75	175.58 ± 5.01	180.28 ± 6.76	153.61 ± 1.60	149.88 ± 3.52	146.65 ± 9.09

*Zero day refers to measurements 6 hrs after slaughter. Data are expressed as an average of 3 cattle and S.E.

mortem storage and described that three endopeptidases were involved in post-mortem proteolysis: 1) the lysosomal cathepsins; 2) the multicatalytic protease complex (MCP); 3) the calpains. However, he concluded that cathepsins and MCP do not play a major role in post-mortem proteolysis and that calpain proteolytic system is responsible for post-mortem proteolysis that results in "meat tenderization". As the authors¹²⁾ reported previously, peptide contents in the ES muscle homogenate increased more than in the control homogenate even after addition of 1 mM EDTA which is an inhibitor of calpains and is an activator of cathepsin B and L. The pH of meat treated with ES was below 6.0, 2 hrs after slaughter, and homogenates used in both experiments had a pH of 5.6. These results show that proteolysis in post-mortem muscles are taken place by not only calpains but also cathepsins.

Free amino acid content: Table 2 shows the total amount of 18 free amino acids in the homogenate and the sarcoplasm stored at 2°C until 21 days after slaughter. The total amount of free amino acids increased each day during storage; values were 103.3, 104.1 and 107.3 mg in the control, the ES 30 and the ES 60 homogenates six hours after slaughter, and 175.6, 175.6 and 180.3 mg per 100 g meat 21 days after slaughter, respectively. There was no significant difference between the control and the ES homogenates. In our previous report¹²⁾, amino acid contents were greater than those of the present experiment. This reason is due to the method of analysis; i.e. at that time, amino acid content was measured with ninhydrin positive materials but this time, 18 free amino acids were summed using an amino acid analyzer.

The total amount of free amino acids also

Table 3. Free amino acid composition of the control and the ES homogenates (mg per 100 g meat)

Amino acid	0 day after slaughter			2 days after slaughter		
	Control	ES 30	ES 60	Control	ES 30	ES 60
Asp	0.57±0.03	0.80±0.12	0.68±0.01	0.85±0.12	1.05±0.17	1.04±0.06
Thr	2.94±0.13	2.84±0.08	2.82±0.08	3.01±0.09	2.95±0.16	3.13±0.10
Ser	4.26±0.12	4.21±0.35	4.08±0.27	4.43±0.15	4.60±0.30	4.55±0.35
Asn	4.94±0.36	4.92±0.33	4.31±0.19	5.06±0.29	5.63±0.57	5.34±0.22
Glu	18.44±1.53	21.43±2.01	25.18±3.11	16.71±1.07	24.89±3.18	25.26±3.58
Pro	2.43±0.19	2.60±0.07	2.22±0.14	2.28±0.19	2.59±0.04	2.27±0.18
Gly	8.08±0.10	7.13±0.72	7.23±0.64	7.77±0.10	7.05±0.49	7.57±0.82
Ala	35.21±0.83	33.16±1.14	34.41±2.96	33.83±0.61	31.03±0.68	34.48±4.24
Val	3.55±0.24	3.53±0.31	3.17±0.17	3.70±0.19	3.86±0.45	3.89±0.08
Cys	1.38±0.29	1.14±0.19	0.89±0.17	1.30±0.27	0.76±0.08	1.53±0.29
Met	0.43±0.09	0.48±0.02	0.45±0.03	1.04±0.14	1.14±0.11	1.27±0.07
Ile	1.77±0.28	1.88±0.12	1.80±0.01	2.48±0.09	2.53±0.17	2.69±0.14
Leu	3.24±0.16	3.14±0.25	3.01±0.09	4.11±1.14	4.27±0.38	4.42±0.07
Tyr	1.95±0.14	1.91±0.14	2.03±0.09	2.45±0.13	2.43±0.18	2.70±0.07
Phe	1.51±0.06	1.59±0.06	1.71±0.04	2.68±0.03	3.07±0.16	3.14±0.04
Lys	3.84±0.15	4.13±0.42	3.88±0.33	4.37±0.31	4.61±0.53	4.36±0.63
His	4.67±0.26	4.67±0.22	5.34±0.10	4.76±0.32	5.54±0.39	5.03±0.34
Arg	3.95±0.39	4.55±0.49	4.13±0.06	5.16±0.34	4.76±0.42	4.42±0.12
Total	103.25±2.34	104.11±2.85	107.34±6.93	105.99±1.73	112.77±4.36	117.08±9.73

Zero day refers to measurements 6 hrs after slaughter. Data are expressed as an average of 3 cattle and S.E.

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increased in the stored sarcoplasm, although it was less than that of the stored homogenate. The total amount in the control and the ES sarcoplasm ranged between 146.7–153.6 mg per 100 g meat 21 days after slaughter. Increments of free amino acids measured 50.4, 45.8 and 39.3 mg during storage for 21 days in the control, the ES30 and the ES60 sarcoplasm, respectively. These values corresponded to 69.7, 64.0 and 53.9% of the values in the case of homogenate storage, respectively. These results indicate that increased free amino acids originate mainly from peptides or sarcoplasmic proteins in the sarcoplasm rather than in the myofibrillar proteins. Although ES treatment produced much peptides from the both homogenate and sarcoplasm (Table 1), it had no effect on the total amount of free amino acids in the ES homogenates or the ES sarcoplasm.

OKAYAMA *et al.*¹⁴⁾ reported the increase of free amino acids in *M. Semimembranosus* during the

conditioning of Japanese black cattle. The total amount of 18 free amino acids measured 68.5, 218.7, 258.0 and 269.8 mg per 100 g meat 0, 7, 14 and 21 days after slaughter, respectively. The amount on day 0 was lower than that of this experiment, but amounts at 7, 14 and 21 days were much higher than those of this experiment.

Free amino acid composition in the both homogenate and sarcoplasm is shown in Table 3, 4 and 5. Almost all free amino acids increased during storage of the control and the ES homogenates except Cys, Pro and Gly. Cys did not increase during storage and increment of Pro was little. Although Gly had the third highest content of the 18 free amino acids on day 0, growth was small and Gly became the forth-ranked amino acid in the control and the ES homogenates 21 days after slaughter. On the other hand, Leu increased greatly during storage and became the third highest content

Table 4. Free amino acid composition of the control and the ES homogenates (mg per 100 g meat)

Amino acid	7 days after slaughter			21 days after slaughter		
	Control	ES 30	ES 60	Control	ES 30	ES 60
Asp	1.41± 0.19	1.60± 0.26	1.83± 0.24	2.44± 0.29	2.24± 0.16	2.55± 0.21
Thr	3.65± 0.13	3.28± 0.06	3.57± 0.31	5.52± 0.20	4.77± 0.08	4.81± 0.18
Ser	5.66± 0.07	5.12± 0.40	5.70± 0.55	8.47± 0.24	7.57± 0.49	7.42± 0.34
Asn	5.82± 0.38	6.60± 0.58	6.61± 0.56	8.42± 0.07	8.89± 0.76	8.95± 0.20
Glu	17.83± 0.82	25.07± 3.12	29.57± 4.59	23.34± 1.42	31.07± 3.86	32.04± 4.08
Pro	2.76± 0.09	2.67± 0.11	2.28± 0.26	4.20± 0.09	3.72± 0.23	3.33± 0.51
Gly	8.12± 0.39	7.02± 0.78	8.22± 0.89	9.87± 0.50	8.66± 0.85	9.18± 0.88
Ala	34.66± 1.20	29.63± 1.57	35.90± 3.86	40.52± 0.40	35.48± 1.13	38.87± 3.84
Val	4.79± 0.36	4.57± 0.32	5.01± 0.20	7.57± 0.45	7.33± 0.26	7.04± 0.12
Cys	1.57± 0.22	1.07± 0.18	1.32± 0.17	1.39± 0.26	1.49± 0.05	1.48± 0.32
Met	2.83± 0.25	2.38± 0.26	2.66± 0.19	5.45± 0.78	5.25± 0.16	5.17± 0.43
Ile	3.78± 0.03	3.75± 0.10	4.21± 0.24	6.33± 0.45	7.02± 0.15	7.57± 1.12
Leu	6.30± 0.18	6.39± 0.19	7.01± 0.32	11.53± 1.08	12.62± 0.11	12.71± 1.09
Tyr	4.09± 0.13	3.62± 0.24	3.96± 0.20	7.83± 0.54	7.24± 0.24	6.94± 0.41
Phe	4.52± 0.15	4.69± 0.07	5.17± 0.26	8.49± 0.75	9.00± 0.03	8.89± 0.47
Lys	5.16± 0.34	5.32± 0.64	5.38± 0.51	8.62± 0.19	8.77± 0.41	8.97± 0.35
His	5.36± 0.41	5.39± 0.21	6.03± 0.30	6.90± 0.20	6.80± 0.08	6.82± 0.12
Arg	5.56± 0.46	5.02± 0.39	5.45± 0.25	8.66± 0.64	7.66± 0.76	7.54± 0.23
Total	123.86± 2.67	122.65± 2.00	139.87± 12.37	175.55± 5.75	175.58± 5.01	180.28± 6.76

Data are expressed as an average of 3 cattle and S.E.

Table 5. Free amino acid composition of the control and the ES sarcoplama (mg per 100 g meat)

Amino acid	7 days after slaughter			21 days after slaughter		
	Control	ES 30	ES 60	Control	ES 30	ES 60
Asp	0.44± 0.18	0.92± 0.10	1.08± 0.01	0.91± 0.06	1.26± 0.06	1.41± 0.18
Thr	3.23± 0.12	2.95± 0.06	3.29± 0.18	3.67± 0.15	3.54± 0.15	3.85± 0.19
Ser	4.73± 0.10	4.95± 0.44	5.15± 0.41	5.88± 0.07	6.00± 0.31	6.07± 0.41
Asn	5.53± 0.40	5.41± 0.30	5.64± 0.38	5.63± 0.16	6.57± 0.54	7.12± 0.67
Glu	30.82± 2.47	29.37± 4.09	29.42± 4.56	47.36± 2.20	33.55± 5.50	30.80± 4.38
Pro	2.69± 0.15	2.38± 0.09	2.37± 0.23	3.08± 0.29	2.92± 0.07	2.88± 0.11
Gly	8.60± 0.14	7.78± 0.84	8.31± 0.75	9.71± 0.12	8.50± 0.75	8.88± 0.85
Ala	30.64± 0.61	33.81± 2.65	37.95± 3.97	31.25± 1.84	37.39± 1.97	35.73± 1.66
Val	4.09± 0.26	4.58± 0.20	4.91± 0.12	5.62± 0.31	6.42± 0.38	6.46± 0.56
Cys	1.90± 0.26	0.87± 0.32	1.33± 0.44	0.70± 0.25	0.65± 0.11	1.53± 0.29
Met	1.05± 0.04	1.36± 0.18	1.63± 0.07	1.92± 0.15	2.73± 0.28	2.61± 0.27
Ile	2.69± 0.12	2.72± 0.11	2.95± 0.01	3.73± 0.21	4.11± 0.16	4.30± 0.61
Leu	4.69± 0.18	4.64± 0.22	4.78± 0.16	7.25± 0.49	7.18± 0.42	6.97± 0.74
Tyr	3.35± 0.29	3.22± 0.19	3.48± 0.08	5.10± 0.41	4.95± 0.23	5.13± 0.57
Phe	2.47± 0.14	2.38± 0.04	2.67± 0.12	4.01± 0.25	4.13± 0.07	4.28± 0.53
Lys	4.83± 0.27	5.11± 0.62	5.19± 0.29	6.04± 0.10	7.19± 0.75	6.98± 0.80
His	4.83± 0.29	5.65± 0.29	5.73± 0.10	6.30± 0.32	6.68± 0.41	6.12± 0.10
Arg	4.40± 0.55	4.99± 0.62	4.48± 0.45	5.47± 0.37	6.09± 0.24	5.52± 0.62
Total	120.97± 3.56	123.08± 6.92	130.35± 10.72	153.61± 1.60	149.88± 3.52	146.65± 9.09

Data are expressed as an average of 3 cattle and S.E.

Fig. 2. Changes in Glu content during the storage of homogenate and sarcoplama. Bars mean S.E.

in the control and the ES homogenates 21 days after slaughter.

A remarkable change was observed in the case of Glu, among the control and the ES homogenates (Table 3 and 4, Fig. 2). The content of Glu increased after ES treatment from day 0, measuring 18.4, 21.4 and 25.2 mg per 100 g meat in the control, the ES30 and the ES 60 homogenates, respectively. This increasing trend was seen until 21 days after slaughter; the content of Glu increased to 23.3, 31.1 and 32.0 mg at 21 days, respectively. Glu content was 2.81 and 21.34 mg in the *M. Semimembranosus* of Japanese black cattle 0 and 7 days after slaughter⁽⁴⁾, and 6.03 and 9.71 mg in beef round 4 and 12 days after slaughter⁽¹³⁾. In the present study, the content of Glu in the ES homogenates were higher than that of the control in this experiment or the these both reports.

Another amino acid which increased due to ES treatment was Phe in the both ES homogenates at 0, 2 and 7 days, although there was little growth. On the contrary, there was a decrease in free amino acids with ES treatment in the case of Pro, Try and Arg in the ES homogenates at 2, 7 and 21 days.

On the other hand, in the stored sarcoplasm, content of almost all free amino acids increased during storage, but was lower than those of the homogenates in storage except Glu, Gly and Ala (Table 5). The content of Glu in the control sarcoplasm increased rapidly during storage and became the highest (47.36 mg per 100 g meat) at 21 days, to a level of twice that of the control at day 0 (Table 3, 5 and Fig. 2). According as increment of Glu in the control sarcoplasm, the content of Ala decreased compared to the control homogenate (Table 3, 4 and 5). It is well known that when Ala is changed to pyruvic acid in the metabolism of cell, Glu is produced by alanine aminotransferase. However, the contents of Glu and Ala in the ES sarcoplasm were not different from those of the ES homogenates. The only

difference between the control homogenate and the control sarcoplasm was the removal of myofibrils. There was no stimulation to proteases by ES treatment. These results suggest following possibility: myofibrils contain inhibitors of such as Glu aminopeptidases or alanine aminotransferase and by removing the myofibrils, these enzymes may work and produce more Glu. However, it is not clear how Glu is produced in the relation of the control sarcoplasm and the ES homogenates, but one can definitely state that ES treatment changes the balance among enzymes, inhibitors and substrates, thereby producing much Glu.

Previously, the author⁽¹¹⁾ reported a high IMP content in ES muscle in Holstein steers, and now the present study shows that ES treatment caused the accumulation of a large content of peptides and Glu. These results indicate that ES treatment improves the favor of beef.

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牛肉ホモジネートおよび筋漿の貯蔵中におけるペプチド および遊離アミノ酸量に及ぼす電気刺激の影響

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電気刺激を行なった牛肉の熟成中におけるペプチドおよび遊離アミノ酸量を知るため、ヘレフォード種去勢肥育牛9頭に対し、40 V, 13.8 Hzで0 (対照区), 30 (ES30区) および60 (ES60区) 秒の電気刺激を行なった。供試筋肉部位は大腿二頭筋を用い、牛肉ホモジネートおよび筋漿画分を調製し、2°Cで屠殺0 (6時間), 2, 7, 14 および21日目まで保存した。ES30およびES60区のホモジネート保存のペプチド量は、屠殺0日目から対照区のものよりも多く、対照区, ES30およびES60区における量は、それぞれ牛肉100g当り、162.5, 192.1 および198.6mgであった。この増加傾向は21日目まで続き、それぞれ356.4, 409.7 および405.5mgとなった。総遊離アミノ酸量は保存日数とともに増加したが、対照区と電気刺激区に差は見られなかった。しかしながら、電気刺激区におけるグルタミン酸量は屠殺0日目から増加しており、21日目には対照区の23.3mg (100g牛肉中) に対して、ES30およびES60区は31.1-32.0mgまで増加した。ペプチド量およびグルタミン酸量を増加させることから、ESは牛肉の呈味性向上に有効であることが認められた。一方筋漿の状態で保存した場合、ペプチドおよび遊離アミノ酸量は増加したが、その増加量は21日目において、ホモジネート保存時のそれぞれ45.8-48.5%および53.9-69.7%に相当した。これらのことから、ペプチドおよび遊離アミノ酸の生成は、筋漿のタンパク質やペプチドと同様に筋原線維タンパク質にも由来していることが分った。

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