Effects of Conditioning on the Content and Behavior of Proteins and Peptides in Sarcoplasm of Japanese Black Cattle

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SUMMARY

Two experiments were conducted to clarify the content and behavior of proteins and peptides in sarcoplasm during conditioning of Japanese Black Cattle. In Experiment I, the influence of conditioning on the content and behavior of proteins in different subcellular sarcoplasmic fractions of beef was investigated. The content of mitochondrial and lysosomal proteins decreased during conditioning. The SDS-PAGE patterns of the conditioned beef samples revealed the components of the cytoplasmic fraction which had changed. In Experiment II, the influence of conditioning on the content and behavior of peptides in the low-molecular weight sarcoplasm fraction and heated soup of beef was investigated. The amount of peptides in the low-molecular weight sarcoplsam fraction and soup increased during conditioning, particularly in the soup. The SDS-PAGE patterns for low-molecular weight components of the sarcoplasm and soup of the conditioned beef samples exhibited the appearance of components below 10,000 daltons. Size exclusion (SEC) HPLC profiles showed some condition sensitive fractions in the low-molecular weight sarcoplasm fraction during conditioning. SEC/HPLC of the low-molecular weight sarcoplasm fraction showed that the first three elution peaks in the stage from 0 to 6 days of conditioning practically disappeared when conditioned for 8 days.

INTRODUCTION

The conditioning of meat involves holding or storage while the indigenous enzymes hydrolyze some of the muscle proteins in order to improve tenderness and flavor. Conditioning of meat is not a new process and has been conducted for many years (LEHMANN, 1907 ; HOAGLAND et al., 1917). Nevertheless, the exact mechanism(s) of tenderization and improvement in flavor during conditioning of meat is still not understood (PEARSON and YOUNG, 1989). This is because conditioning of meat is a very complex phenomenon. In myofibrillar proteins, polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) of myofibrils prepared from conditioned meat shows troponin T to disappear and a number of polypeptides of lower molecular weight to be formed, such as a 30,000 dalton component that is particularly noteworthy (MACBRIDE and PARRISH, 1977 ; PENNY and DRANSFIELD, 1979). However, little is known about changes in sarcoplasmic proteins and peptides during conditioning of meat. Recently, OKAYAMA et al. (1991) investigated that changes in the physical and chemical properties of muscles during conditioning of Japanese Black Cattle, and they reported that sarcoplasmic protein nitrogen in the beef was significantly changed during conditioning.

This study was conducted to elucidate the effects of conditioning on the content and behavior of proteins and peptides in the sarcoplasm of Japanese Black Cattle. A useful indicator of the course of conditioning in the sarcoplasm fraction of beef was sought.

MATERIALS and METHODS

Experiment I.

1. Materials. Semimembranosus muscles from four Japanese Black Cattles approximately 29 month of age were sampled after 3 days slaughter. Muscles stored at 0°C were analysed after 7, 14, 21 and 28 days after slaughter.

2. Extraction and subcellular fractionation of sarcoplasmic proteins. Minced sample was homogenized in 6 volumes of chilled sarcoplasmic-protein-extracting solution at 12,000 rpm for 2 min. The homogenate was centrifuged at 500 x g for 10 min to precipitate myofibrillar and connective tissue fractions. The sarcoplasm fraction was collected as upper layer. The differential centrifugation procedure of ASGHAR et al. (1986) was carried out to separate different subcellular fractions (nuclear, mitochondrial, lysosomal, microsomal consisting of

heavy, medium, and light fractions, and cytoplasmic proteins).

3. Protein measurement. The protein content in each fraction was measured by the modified version of the method of LOWRY et al. (1951), as described by PETERSON (1983).

4. SDS-PAGE. SDS-PAGE was performed by the method of LAEMMLI (1970), using a 12.5% acrylamide separating gel slab (140 x 100 x 1.0 mm) with a 4.5% acrylamide stacking gel. Electrophoresis was conducted at a constant current of 5 mA per slab for about 20 hr at room temperature.

Experiment II.

1. Materials. Semimembranosus muscles from four Japanese Black Cattles approximately 29 month of age were sampled immediately after slaughter. Muscles stored at 4°C were analysed after 0, 2, 4, 6, 8, 10, 14, 17 and 21 days after slaughter.

2. Preparation of low-molecular weight sarcoplasm fraction and heated soup. Minced sample was homogenized in 2 volumes of chilled 0.6% NaCl solution at 15,000 rpm for 2 min. The homogenate was adjusted to pH 6.0 with lactic acid or NaOH solution. The resulting slurry was centrifuged at 20,000 x g for 20 min. The obtained supernatant was used as sarcoplasm. The sarcoplasm was dialyzed against distilled water for 24 hr at 4°C using a Visking tubing. The resulting outside preparation was used as low-molecular weight sarcoplsam fraction. An equal weight of water was added to minced meat, and preparation was homogenized at 15,000 rpm for 2 min. The homogenate was heated in boiling water for 20 min, and then centrifuged at 20,000 x g for 20 min. The resulting solution was used as heated soup.

3. Peptide measurement. The values were measured by the method of LOWRY et al. (1951) and were regarded as the peptide content in each low-molecular weight sarcoplasm fraction and heated soup.

4. SDS-PAGE. SDS-PAGE was performed by the method of LAEMMLI (1970), using a commercial slab gel (Atto Corp, PAGEL, SPU-15S, 90 x 73 x 1.0 mm). Electrophoresis was conducted at a constant current of 20 mA per slab for about 2 hr at room temperature.

5. Size exclusion (SEC) high performance liquid chromatography (HPLC). An isocratic peptide separation system (Tosoh, Inc.) consisting of a Model CCPE-TM pump, Model UV-8010 detector, Model ERC-3512 degasser (Erma) and Model 12 chromatocorder (Sic) was used in this study. Peptides were detected by absorbance at 215 nm. The system mobile phase was 0.1M phosphate buffer, pH7.0. This was pumped at a flow rate of 0.5 ml/min. The column was TSKgel G2000SW-XL, 7.8 mmI.D. x 30 cm (Tosoh,Inc.). Sample injection was at a constant volume of 20 µl.

RESULTS and DISCUSSION

Experiment I.

1. Protein content of different subcellular sarcoplasmic fractions from beef muscles. Table 1 shows that the content of mitochondrial proteins after conditioning for 28 days was significantly ($P<0.05$) lower compared to that after 7 days of conditioning. The content of lysosomal proteins in the beef conditioned for 21 and 28 days was significantly ($P<0.05$) lower than in that conditioned for 7 days. The protein content of other fractions did not significantly change during conditioning. The proteins of mitochondrial and lysosomal fractions may thus possibly be denatured during conditioning. Changes in the protein content of lysosomal

Table 1. Changes in protein content of different subcellular sarcoplasmic fractions during conditioning of beef (mg/100g meat)

Subcellular fraction	Days conditioning 7	14	21	28
Sarcoplasmic fraction	7944	7024	7461	7828
Nuclear plus broken myofibril fraction	449	430	425	308
Mitochondrial fraction	138[a]	121[ab]	107[ab]	91[b]
Lysosomal fraction	110[a]	86[ab]	71[b]	63[b]
Heavy microsomal fraction	133	90	92	81
Medium microsomal fraction	71	86	81	65
Light microsomal fraction	67	78	82	87
Cytoplasmic fraction	6581	6465	6535	6377

a,b, Means in the same column having different superscript are significantly different ($P<0.05$).

fraction were of particular interest with respect to lysosomal enzyme (cathepsins) activity during conditioning. OKAYAMA et al. (1991) reported that sarcoplasmic protein nitrogen in the beef conditioned for 21 days was significantly ($P<0.05$) less than in that conditioned for 7 and 14 days. In this study, this was not noted in the protein content of different subcellular sarcoplasmic fractions during conditioning, the reasons are unknown at the present time.

2. SDS-PAGE patterns. Fig. 1 shows the SDS-PAGE patterns of proteins in the cytoplasmic fraction during conditioning. These numerous protein bands were estimated to be between 65,000 and 25,000 in molecular weight. At least 80% of the sarcoplasmic proteins are normally constituted by the glycolytic enzymes (SCOPES, 1970). McCORMICK et al. (1988) reported that fourteen protein components of porcine sarcoplasmic proteins could be separated and identified by reversed-phase HPLC and SDS-PAGE. The patterns of the SDS-PAGE (Fig. 1) are generally in agreement with the results of McCORMICK et al. (1988). After conditioning for 7 days and 14 days, the bands corresponding to creatine kinase (CK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) had disappeared. All bands of cytoplasmic protein components could

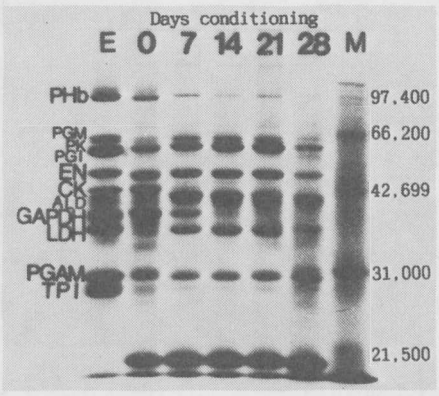

Fig. 1. Changes in the SDS-PAGE of cytoplasmic proteins during conditioning of beef. E, Enzyme preparations; PHb, phosphorylase b; PGM, phosphoglucomutase; PK, pyruvate kinase; PGI, phosphoglucose isomerase; EN, enolase; CK, creatine kinase; ALD, aldolase ; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDH, lactate dehydrogenase ; PGAM, phosphoglycerate mutase; TPI, triose phosphate isomerase; M, molecular marker.

generally be seen in the weak color bands during conditioning for 28 days. But, after that, the bands corresponding to pyruvate kinase (PK), enolase (EN) and phosphoglycerate mutase (PGAM) were detected clearly. CK and GAPDH were unstable toward enzymatic activity at pH 5.5 at 55°C (SCOPES, 1970), and OKAYAMA et al. (1991) reported that CK and GAPDH were heat-unstable proteins based on SDS-PAGE patterns of sarcoplasmic proteins of model sausage during cooking treatment.

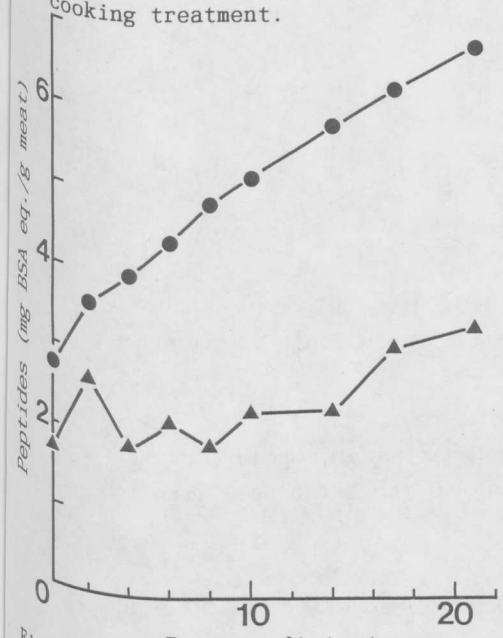

Fig. 2. Changes in peptide content of the low-molecular weight sarcoplasm fraction and heated soup during conditioning of beef. ▲—▲: low-molecular weight sarcoplasm fraction ; ●–●: heated soup.

Experiment II.

1. Peptide content of the low-molecular weight sarcoplasm fraction and heated soup. Fig. 2 shows that the content of the low-molecular weight sarcoplasm fraction and heated soup of beef after conditioning. Peptides in the low-molecular weight sarcoplasm fraction gradually increased during conditioning, except for two days only. It is well known that peptides increased in meat during conditioning (KHAN and BERG, 1964 ; SUZUKI et al., 1967 ; NISHIMURA et al., 1988). NISHIMURA et al. (1988) found peptides in bovine round conditioned at 4°C from 4 to 12 days after slaughter to increase by about 21%, while in this study from 4 to 14 days, they increased by about 25%. Peptides in heated soup increased linearly during conditioning (Fig. 2). NISHIMURA et al. (1988) reported that peptide levels in the soup of beef conditioned for 12 days were lower in that after conditioned for 4 days, but in pork and chicken, peptide levels were higher in soup prepared from meat after conditioning than that before conditioning. The results for beef were at variance with those of the present study, the reason being unclear. Increase in peptide levels in samples by heating appears to be caused by the action of endopeptidases on pro-

teins at the start heating (NISHIMURA et al., 1988).

2. SDS-PAGE patterns. Fig. 3 shows the SDS-PAGE patterns of sarcoplasmic protein during conditioning. Bands below 10,000 daltons were of particular interest. These were slightly apparent in samples during conditioning. After conditioning for 2 days, a band corresponding to about 5,000 daltons could be detected clearly. Fig. 4 shows the SDS-PAGE patterns of heated soup during conditioning. Bands corresponding to about 4,000 daltons became gradually apparent in samples during conditioning.

3. SEC/HPLC chromatogram. Fig. 5 shows SEC/HPLC chromatograms of the low-molecular weight sarcoplasm fraction during conditioning. Seven identifiable component peaks (215 nm absorbance) showed in sample conditioned for 0 day. DAVIS and ANDERSON (1984) examined SEC/HPLC chromatograms of heated water soluble bovine muscle proteins, but little is known about SEC/HPLC chromatograms in the low-molecular weight sarcoplasm fraction during conditioning. SEC/HPLC of the low-molelcular weight sarcoplasm fraction showed that the first three elution peaks in the stage from 0 to 6 days of conditioning practically disappeared when conditioned

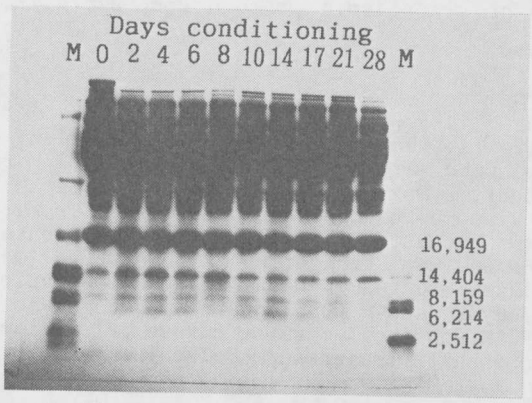

Fig. 3. Changes in the SDS-PAGE of sarcoplasm fraction during conditioning of beef. M, molecular marker.

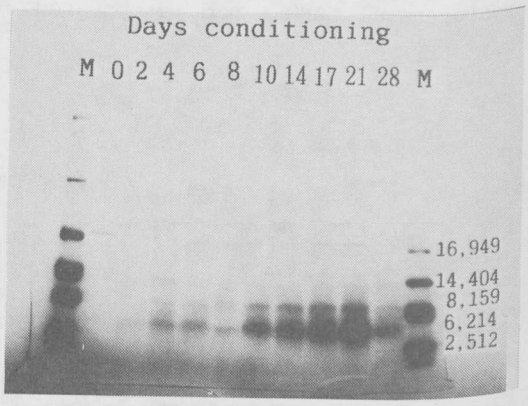

Fig. 4. Changes in the SDS-PAGE of heated soup during conditioning of beef. M, molecular marker.

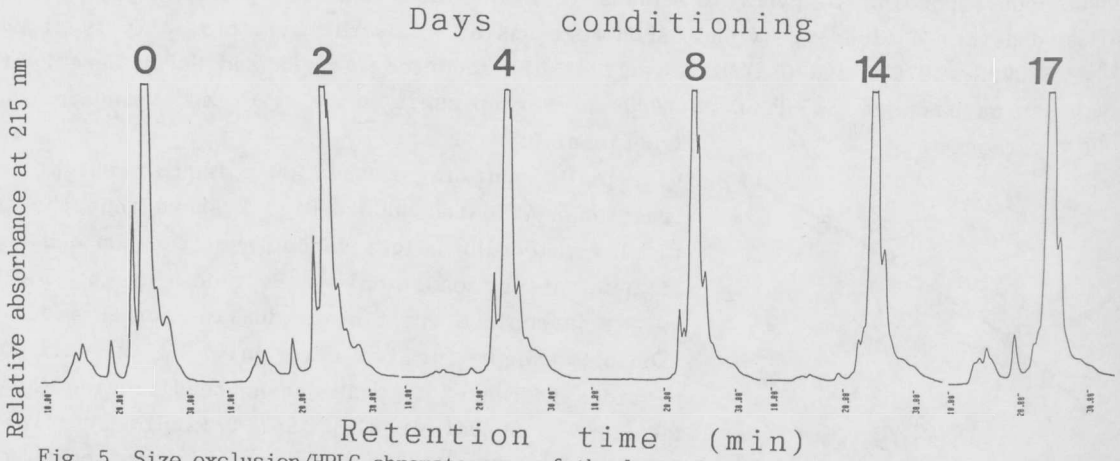

Fig. 5. Size exclusion/HPLC chromatograms of the low-molecular weight sarcoplasm fraction during conditioning of beef.

for 8 days. Peaks similar to these could be seen again conditioning for 17 days. These results should help to provide some clarification of the degradation of muscle proteins and serve as a reliable indicator of the course of conditioning in the sarcolpasm fraction of beef.

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