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Meat Science 56 (2000) 311–318

MEAT SCIENCE

www.elsevier.com/locate/meatsci

Meat tenderization by proteolytic enzymes after osmotic dehydration

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Received 29 April 1999; accepted 15 May 2000

Abstract

The treatment of proteolytic enzymes is one of the popular methods for meat tenderization. In this case, it is very important how to introduce the enzymes into the meat cut. This paper describes meat tenderization by dipping the meat cut in a solution containing proteolytic enzymes after contact-osmotic dehydration. After the dehydration of each piece of meat from culled cow for 18 h by contact-dehydration sheet, each sample was dipped for 3 h in a solution containing papain or proteinases from *Aspergillus* traditionally used for soysauce production in Japan. It was stored at 3~4°C for 24, 48 and 168 h, and subjected to texture measurement, sensory evaluations, biochemical analysis and histological observations. The penetration efficiency of the enzyme solution (of around 80%) after the contact-osmotic dehydration seemed to be sufficient. A marked decrease in hardness by texture measurements was observed in the meats treated with proteolytic enzymes and higher sensory scores for tenderness were observed in the meats treated with enzymes as compared with the untreated meat. The papain-treated meat received the highest score in tenderness, but the scores given to juiciness and taste were lower than that of the control. The rapid increases of the fragmentation of myofibrils from the enzyme-treated meat were observed at first 24 h of storage as compared with that of the control. Remarkable degradation of myosin molecule in the myofibrils from the enzyme-treated meats was observed on SDS-PAGE profiles. Considerable degradation of myofibrilar structure especially due to proteolytic removal of Z-lines, was observed among the myofibrils from enzyme-treated meats by electronmicroscopy. The remarkable deformation and disruption of honeycomb-like structure of endomysium were also observed in the meats treated with enzymes. From these results, it was shown that treatment after osmotic dehydration, was effective in tenderizing.

Keywords: Meat tenderization; Proteolytic enzymes; Osmotic dehydration; Myofibrils; Intramuscular connective tissue

1. Introduction

Of all the attributes of eating quality of meat, tenderness is rated as the most important by the consumer. There are several means of tenderizing meat, chemically or physically. Treatment by proteolytic enzymes is one of the popular methods for meat tenderization. In this case, the introduction of the enzyme into meat is very important. There are three methods introducing the proteolytic enzymes into meat cuts post mortem, such as dipping in a solution containing proteolytic enzymes, pumping enzyme solution into major blood vessels of meat cut and rehydration of the freeze-dried meat in a solution containing a proteolytic enzyme. The first two methods are somewhat unsatisfactory, since they overtenderize the surface, producing a mushy texture, and, since they are unable to penetrate within the meat, leaving the interior unaffected (Lawrie, 1998). The rehydration of the freeze-dried meat showed a much better distribution of enzymes than did dipping or perfusion, but was still not ideal, and the setting up of a freeze-dryer is required. Instead of introducing enzymes into meat post mortem, pre-slaughter injection of the enzymes into live animals was developed and proved to be the most effective method of introducing the enzymes into meat. Since the appearance of the patent by Beuk, Hinsdale, Savich, Goeser and Hogan (1959), many papers describing this method have been published (Brooks, Klasing & Regenstein, 1985; Kang & Warner, 1974; Suzuki, Shimakura, Miki, Shimizu, Koyama, Saito &

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0309-1740/00/$ - see front matter
PII: S0309-1740(00)00060-7

Ikeuchi, 1990). In this case, it is important to select an enzyme suitable for live animals. If an unsuitable enzyme is injected, it may cause shock to the animal, resulting in the production of low-grade meat. This method is not easy, because handling live animals requires a skilled operator. Osmotic dehydration with a contact dehydration sheet (Numamoto & Kasai, 1983) is a simple method for dehydration of meat. No special equipment or a skillful operator is required.

This paper describes meat tenderization by dipping the meat cuts in a solution containing proteolytic enzyme after osmotic dehydration.

2. Materials and methods

Lean meat was excised from the shoulder part of a culled-cow carcass 2 days after slaughter and stored at −25°C. As required, it was tempered overnight in a cold room (3∼4°C) and cut into small pieces (30×30×20 mm). Four small pieces of meat were prepared for each experiment. Each experiment shown in this paper was repeated four or five times.

2.1. Contact-dehydrating sheet

Pichit, a contact-dehydrating sheet (US patent 4383376, product of Showa Denko Co. Ltd., Tokyo) was used. The dehydrating sheet consists of a high osmotic pressure substance (e.g. thick malt syrup, glucose, sorbitol, etc.), a polymeric water absorbent (e.g. acrylic acid salt, acrylic acid esters, polyvinyl pyridine, etc.) and a hydrophilic alcohol (e.g. propylene glycol, glycerol, etc.) which are integrally covered with a semipermeable membrane allowing selective permeation of water.

2.2. Dehydration and enzyme treatment

Each piece of meat covered with a cellophane sheet was placed between contact-dehydrating sheets i.e. the contact sheets were placed on two sides of each meat cut, and stored for 18 h in a cold room. After the dehydration, each sample was dipped for 3 h in a cold room in 2 volumes (w/w) of solution containing proteolytic enzymes, such as papain (Sigma Co., USA) and proteinases from *Aspergillus sojae* and *Aspergillus oryzae* (Kikkoman Co., Tokyo), which are traditionally used for soysauce production, An untreated sample (control) was dipped in deionized water instead of an enzyme solution after osmotic dehydration. After being removed from the solution, each sample was stored for 24, 48 and 168 h in a cold room and then subjected to various kinds of analyses and electron microscopic observation. The concentration of enzyme solution was 0.1% for papain and 1% for proteinases from *Aspergillus*.

2.3. Texture measurement

Meat tenderness was measured by Rheometer NRM-2002 (Fudoh Co. Ltd., Tokyo) with a conical plunger according to the procedure described by Okabe (1979). The penetration of the plunger was set parallel to the fiber direction of the meat.

2.4. Sensory evaluation

The samples treated with proteolytic enzymes were stored in a cold room for 3 days and subjected to sensory evaluation. Each side of the samples were cut into small pieces (20×20×20 mm) were grilled at 200°C for 30 s. The panel members (11 participants) were students and staff of the University of Niigata, but were not trained in the sensory analysis of meat. Panel members were asked to fill in a questionnaire, containing six questions, on appearance. tenderness, juiciness, bitterness, flavor, and taste. Each trait except bitterness was scored in a 7-point scale from −3 to +3: very poor, fairly poor, a little poor, average, a little good, fairly good, and excellent. In the case of bitterness, a 7-point scale from −3 to +3 means an increase of bitterness. In this test, the scores were evaluated in comparison to known control. Average (0 score) means the score evaluated for untreated sample (control).

2.5. Preparation of myofibrils and fragmentation index

Myofibrils were made from each muscle according to the procedures described by Busch, Stromer, Goll and Suzuki (1972). The ground muscle was suspended in 6 volumes (w/v) of 50 mM Tris–HCl buffer (pH 7.6) containing 100 mM KCl and 5mM EDTA by using a Waring Blendor for 15 s. The myofibrils were sedimented in 1000 *g* for 10 min and suspended again in 5 volumes of the same buffer by use of a Waring Blendor for 10 s. The resuspended myofibrils were sedimented at 1000 *g* for 10 min, and the resuspension–sedimentation process was repeated three more times. After the fifth wash, the myofibrils suspended in the same buffer were passed through a 20 mesh nylon net to remove connective tissue. The strained myofibrils were sedimented at 1000 *g* for 10 min washed three times in 1000 mM KCl and finally suspended in 100 mM KCl and 1 mM NaN_3. After adjusting the protein concentration to 0.5 mg/ml of 100 mM KCl, turbidity at 540 nm of the solution was measured as fragmentation index (Moller, Vestergaard & Wismer-Pedersen, 1973) by Hitachi Spectrophotometer U-2000 (Hitachi Co. Ltd., Tokyo).

2.6. SDS-PAGE

The myofibrils were sedimented by centrifuging at 5000g for 10 min, and were solubilized in 0.01 M

sodium phosphate buffer (pH 7.0) containing 5% SDS and 1% 2-mercaptoethanol in boiling water for 2 min, with subsequent centrifugation at 10,000 *g* for 15 min. The clear supernatant was analyzed by SDS-PAGE, which was conducted according to the procedure described by Laemmli (1970) with a slight modification. The electrophoresis was carried out on 7.5% polyacrylamide [bisacrylamide/acrylamide, 1:20 (w/w) slab gel (70×90×1 mm) containing 0.1% SDS at constant current of 20 mA for 1.5 h, and then the gels were stained with Coomassie Brilliant Blue R-250. The SDS-PAGE profiles of the myofibrils were scanned densitometrically with a Toyo DMU-33C Digital Densitorol (Toyo Kagaku Sangyo, Tokyo) with a 610 nm filter.

2.7. Electronmicroscopic studies

After centrifugation at 4000 *g* for 10 min, the myofibrils were fixed for transmission electronmicroscopic (TEM) examination. The TEM observation was carried by the procedure of Suzuki, Saito, Sato, and Nonami (1978). Specimens for scanning electronmicrograph (SEM) of intramuscular connective tissue were prepared by the cell-maceration method of Ohtani, Ushiki, Taguchi and Kikuta (1988). Briefly, small pieces (5×5×5 mm) from the control or enzyme-treated muscles were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0 for 1 day. The pieces were immersed in 10% NaOH for 7 days, the NaOH solution being replaced every day by a fresh one and then rinsed in distilled water for 5 days at room temperature. Then the pieces were put in 1% tannic acid for 3 h, rinsed in distilled water for several hours, and post-fixed in 1% osmium tetraoxide–0.1 M phosphate buffer, pH 7.0 for 1 h. The specimens were dehydrated through graded ethanol, freeze-fractured with a razor blade in liquid nitrogen, and dried by the τ -butyl alcohol freeze-drying method (Inoue & Osatake, 1988). The dried specimens were coated with gold and examined using a SEM ABT-55 (Akashi Beam Technology Co., Tokyo) with an accelerating voltage of 10 kV.

2.8. Protein concentration

The protein concentration was measured by the biuret method (Gornall, Bardawill, & David, 1949) standardized against crystalline bovine serum albumin.

3. Results and discussion

3.1. Dehydration and absorption of enzyme solution

The absorption ratio of enzyme solution [absorption of enzyme solution (ml)/water removed in dehydration (ml) × 100] is shown in Table 1. As shown in the table, the absorption ratio was around 78% of the water removed in dehydration. Differences between absorption ratio were not observed among the enzyme solutions. The penetration efficiency of enzyme solution after the contact-osmotic dehydration of meat seems to be sufficient.

The absorption ratio almost linearly increased with the increase of dipping time up to 3 h, then gradually increased and reached about 90% at 5 h of dipping, but the fading of meat colour due to the release of myoglobin from meat into enzyme solution increased markably over 3 h of dipping (data not shown). Therefore, 3 h of dipping time was chosen in present experiment.

3.2. Texture measurement

The changes in the relative hardness of enzyme-treated meat as expressed as a percentage of that of the control (untreated meat) stored for 24 h is shown in Fig. 1. The decrease in the relative hardness was observed during storage irrespective of the enzyme treatment or

Table 1
Absorption ratio of enzyme solution[a]

Enzymes	Absorption ratio (%) [absorption of enzyme solution(ml)/water removed in dehydration (ml)] ×100
Control	78.13±1.38
Papain	78.20±4.81
Aspergillus sojae	76.81±3.93
Aspergillus oryzae	79.28±2.16

[a] The results shown were obtained by repeated experiments with five different samples.

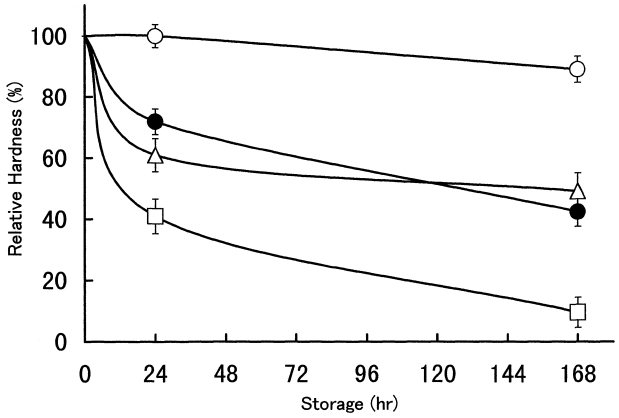

Fig. 1. Changes in hardness of enzyme-treated meats. The samples treated with proteolytic enzymes after osmotic dehydration were stored for 24, 48 and 168 h at 3~4°C, and then subjected to texture measurements. The hardness was measured at five places of each sample. The relative hardness was expressed as a percentage of the control (stored for 24 h without enzyme treatment). The result shown in the figure is obtained by repeated experiments for five different samples. Standard deviations are given by vertical through means. ○- control; -□- treated with papain; -●- treated with proteinase from *A. sojae*. -△- treated with proteinase from *A. oryzae*.

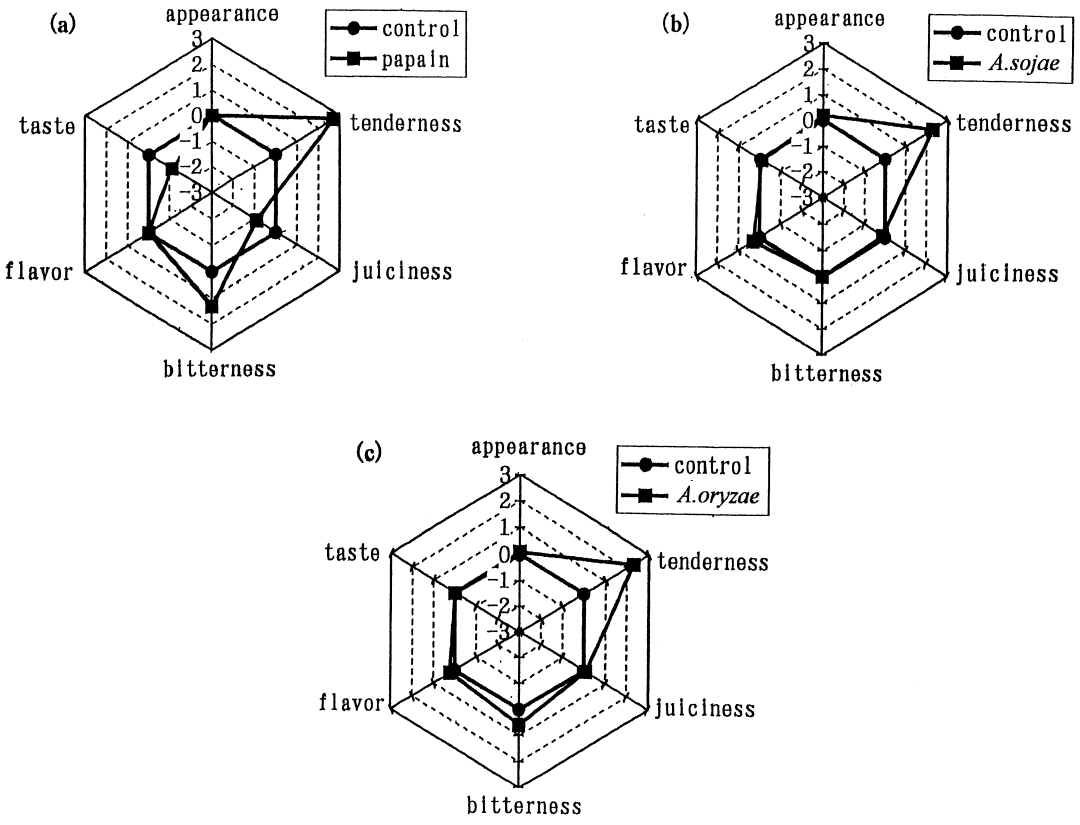

Fig. 2. Sensory evaluations of enzyme-treated meats. The samples treated with proteolytic enzymes after osmotic dehydration were stored for 3 days at 3~4°C and subjected to sensory evaluation. The results shown in figures are representative of those obtained by repeated experiments for four different samples. The details of the evaluation are shown in the text: (a) treated with papain; (b) treated with proteinase from *A. sojae*; (c) treated with proteinase from *A. oryzae*.

not. However it happened more rapidly and completely in the enzyme-treated meat. Among the enzymes tested in this experiment the meat tenderizing activity of papain was the best and no difference in the activity was observed between the proteinases from *A. sojae* and *A. oryzae*.

3.3. Sensory evaluations

The results of the sensory evaluations of grilled meats treated with enzymes are shown in Fig. 2. The papain-treated meat received the highest score in tenderness, but the scores given to juiciness and taste are lower than that of the control. The panel also gave high score for bitterness. (Fig. 2a). The meats treated with proteinases from *A. sojae* and *A. oryzae* received higher scores in terms of tenderness as compared with that of control (Fig. 2b and c). A difference in the scores of meat treated with proteinases from *A. sojae* and *A. oryzae* was not observed, except a slightly higher bitterness score given to meat treated with proteinase from *A. oryzae*.

The sensory evaluation score for tenderness is in good agreement with the results of texture measurement. The

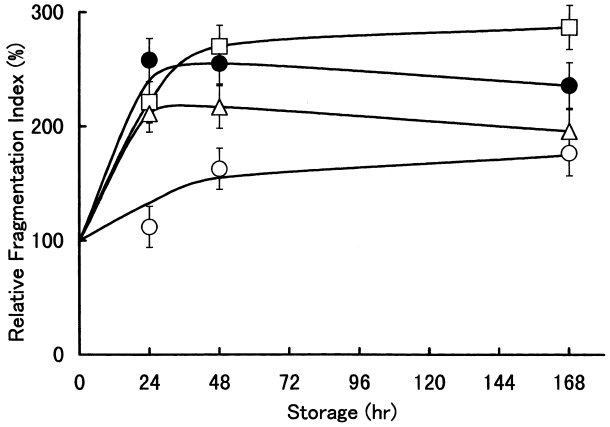

Fig. 3. Changes in the fragmentation index of myofibrils prepared from enzyme-treated meats during storage. The samples treated with proteolytic enzymes after osmotic dehydration were stored for 24, 48 and 168 h at 3~4°C. The relative fragmentation indexes were expressed as a percentage of that of the control (untreated) myofibrils prepared from the meat immediately after thawing. The results shown in figure were obtained by repeated experiments with five different samples. Standard deviations are given by vertical through means. -○- control; -□- treated with papain; -●- treated with proteinase from *A. sojae*. -△- treated with proteinase from *A. oryzae*.

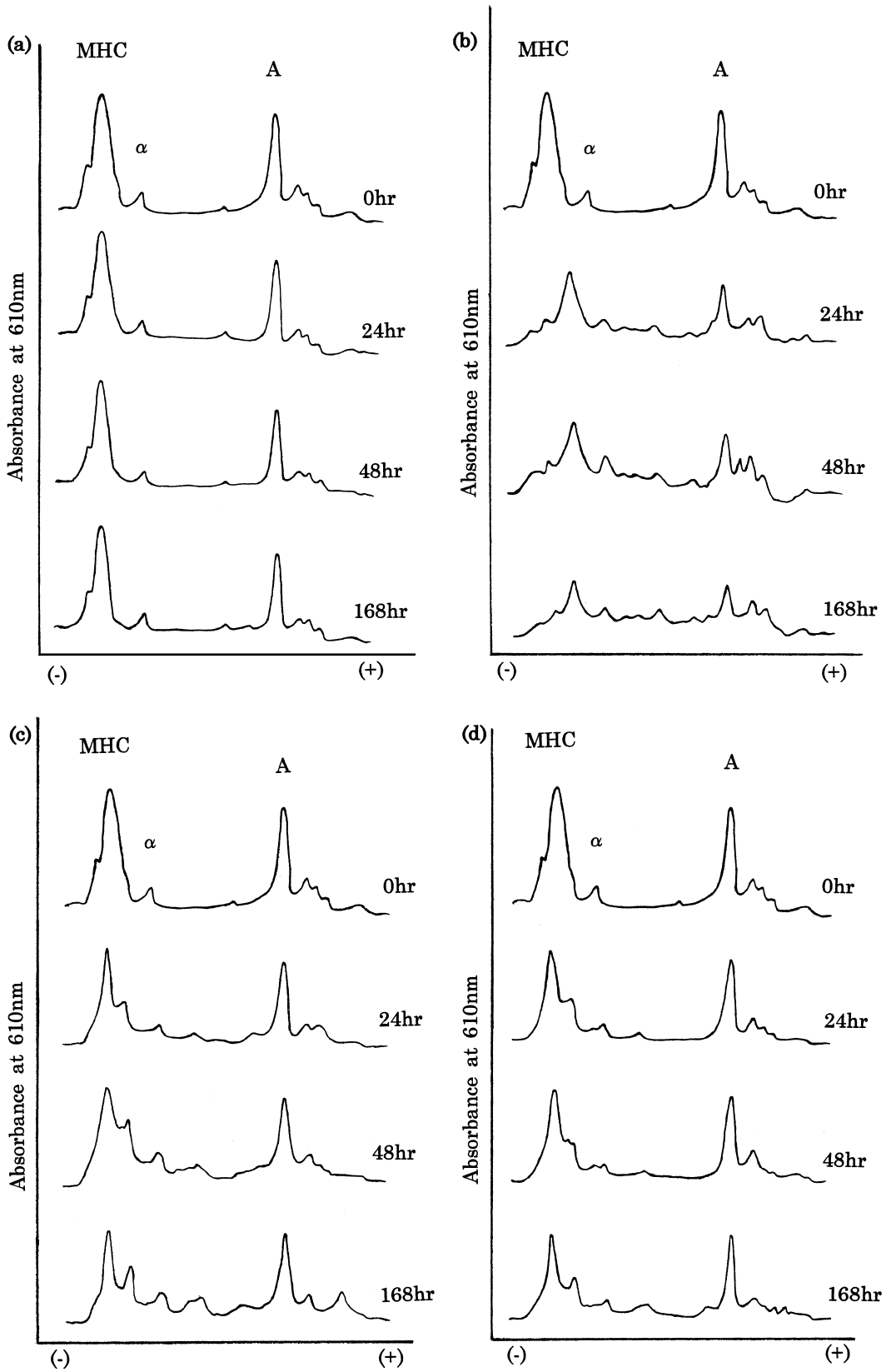

Fig. 4. Changes in SDS-PAGE profile of the myofibrils prepared from enzyme-treated meats during storage. The samples treated with proteolytic enzymes were stored for 24, 48, and 168 h at 3~4°C. The SDS-PAGE profiles were scanned densitometrically. The details of SDS-PAGE analysis were shown in the text. MHC, myosin heavy chain; α, α-actinin; A, actin (a) control; (b) treated with papain; (c) treated with proteinase from *A. sojae*; (d) treated with proteinase from *A. oryzae*.

bitterness of the papain-treated meat is probably due to the appearance of bitter peptides from proteolytic degradation of meat protein. The increase of bitterness, and the decrease of juiciness and taste are problems with using papain as a meat tenderizer. On the contrary, the proteinases from *A. sojae* and *A. oryzae* seem better as a meat tenderizer.

3.4. Fragmentation of myofibrils

The changes in the relative fragmentation of myofibrils prepared from enzyme-treated meat as expressed as a percentage of that of the control (untreated meat) immediately after thawing is shown in Fig. 3. As compared with the myofibrils from the control, the rapid increases of the fragmentation of myofibrils from the enzyme-treated meats were observed at first 24 h of storage. The relative fragmentation ratio of the myofibrils, treated with the proteases from *A. sojae*, papain and *A. oryzae* reached about 240, 200 and 190%, respectively. After that the gradual increases of the fragmentation were observed in the myofibrils treated with papain and proteinases from *A. oryzae* up to 48 h of storage. From 48 to 168 h of storage, a slight increase and decrease of the fragmentation was observed in the myofibrils treated with papain and *Aspergillus* proteases,

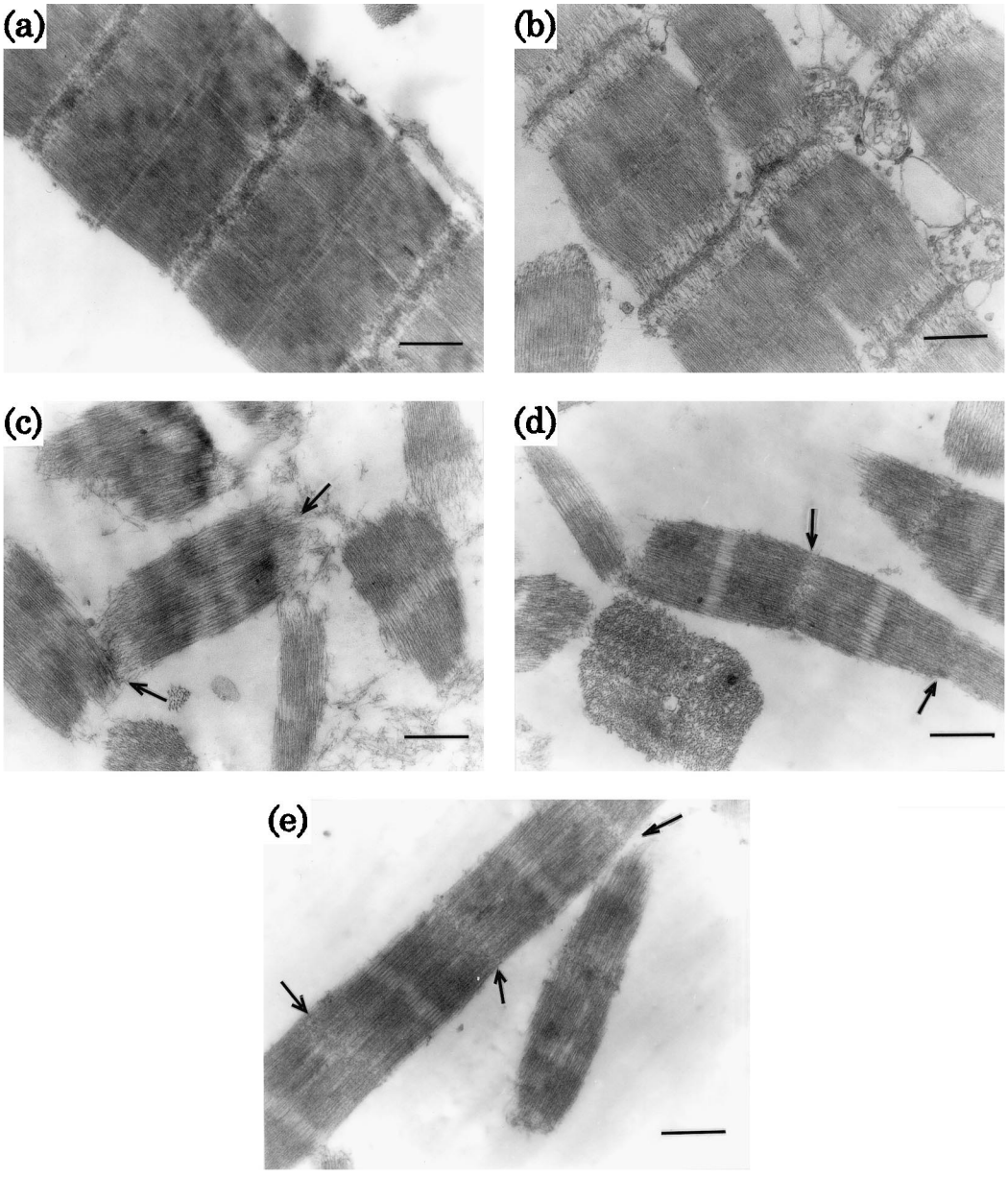

Fig. 5. Transmission electronmicrographs of myofibrils prepared from the enzyme-treated meats. The myofibrils were prepared from the meats treated with proteolytic enzymes after osmotic dehydration and stored at 24 h at 3~4°C, fixed with 3.0% glutaraldehyde and 1% osmium tetroxide, and subjected to TEM examination. The arrows mark the original positions of the Z-lines. Each scale mark indicates 0.5 μm: (a) control; (b) control stored for 168 h; (c) treated with papain; (d) treated with proteinase from *A. sojae*; (e) treated with proteinase from *A. oryzae*.

respectively. In the case of control, gradual increase of the fragmentation was observed throughout the storage, but the ratio of the fragmentation was always lower than that of the enzyme treated myofibrils at any stage of the storage. (especially, at first 48 h storage, the fragmentation ratio of the control was about half of that of the enzyme-treated myofibrils).

These results are in good agreement with that of texture measurements (Fig. 1). The acceleration of fragmentation of myofibrils from the meats treated with proteolytic enzymes is one of the reasons of the meat tenderization as obtained in the texture measurement.

3.5. SDS-PAGE profile of myofibrils

The changes in the profile of densitometric scans of SDS-PAGE gels are shown in Fig. 4. Changes in the constituents of myofibrils from control meat were not observed during the storage (Fig. 4a). The remarkable degradation of myosin heavy chain into proteins with molecular mass of 140 and 90 kDa were observed in the myofibrils treated with papain during storage (Fig. 4b). The decrease of actin, loss of α-actinin, and the appearance of further degradative products with lower molecular mass were also observed. In the myofibrils from the meat treated with proteinase from *A. sojae*, the degradation of myosin heavy chain into proteins with molecular mass of 160, 130, 90, and 75 kDa was different from papain treatment. The loss of α-actinin and the appearance of degradative products with lower molecular mass were also observed as it was for the papain-treated sample (Fig. 4b and c) In the myofibrils from the meat treated with proteinase from *A. oryzae*, the degradation of myosin heavy chain was observed to be the same as with the other protease. However, the degree of the degradation was lower than that of the myofibrils treated with the other protease (Fig. 4d). The degradation of actin was not observed in the myofibrils treated with proteinases from *Aspergillus*.

3.6. Ultrastructures of myofibrils

The ultrastructures of the myofibrils from the enzyme-treated meats stored for 24 h in cold room are shown in Fig. 5. No structural changes were observed in the myofibrils from untreated meat (control) during storage up to 168 h (Fig. 5a and b), whereas remarkable changes in the ultrastructure were observed in every myofibrils from the enzyme-treated meats stored at 24 h (Fig. 5c–e) and the myofibrils with intact structure were not found among the myofibrils prepared from the enzyme-treated meats. The proteolytic degradation of myofibrilar structure, especially degradation of thin filament due to the proteolytic removal of Z-line, may cause fragmentation of myofibrils, resulting in the tenderization of meat. The action of the proteinase from

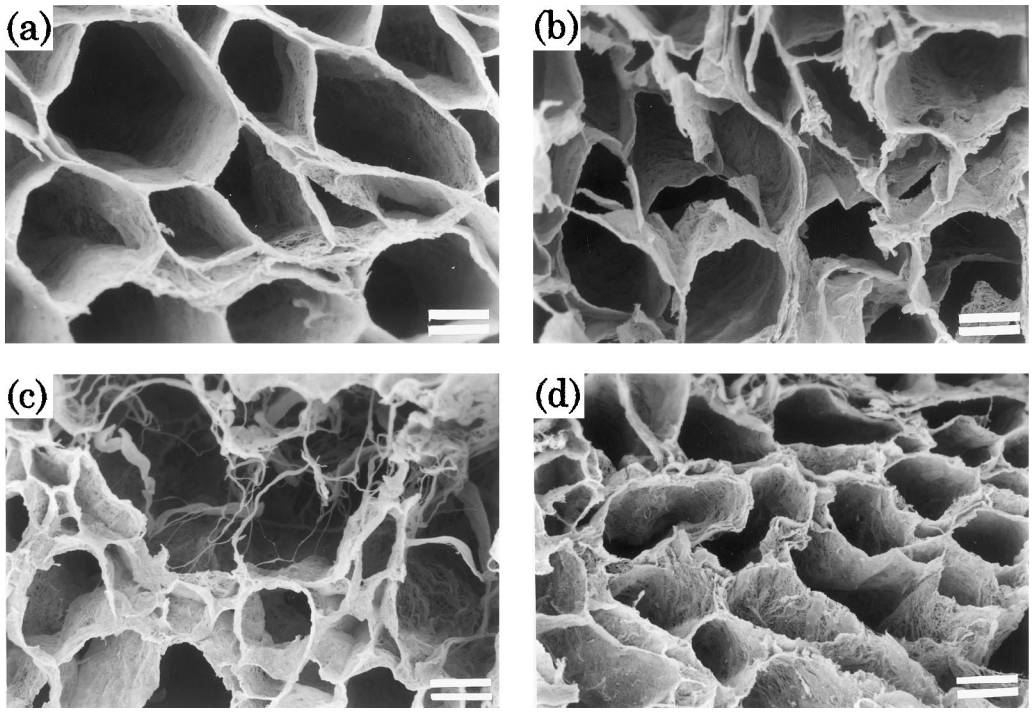

Fig. 6. Scanning electronmicrographs of intramuscular connective tissue prepared from the enzyme-treated meats. The intramuscular connective tissues were prepared from the meats treated with the proteolytic enzymes after osmotic dehydration and stored for 24 h at 3~4°C. Each scale mark indicates 20 μm. (a) control; (b) treated with papain; (c) treated with proteinase from *A. sojae*; (d) treated with proteinase from *A. oryzae*.

A. oryzae on the myofibrils (Fig. 5e) seems to be weaker than that of the other two enzymes. This result is in good agreement with that observed in the fragmentation index (Fig. 3).

3.7. Ultrastructure of intramuscular connective tissue

Scanning electronmicrographs of the intramuscular connective tissue in the meats treated with proteolytic enzymes are shown in Fig. 6. The remarkable deformation and disruption of honeycomb-like structure of endomysium were observed at 24 h of storage as compared with that of the untreated meat. The action of the proteinase from *A. oryzae* on the intramuscular connective tissue seems to be lower than for the other two enzymes. Disruption of the structure of intramuscular connective tissue is another reason for meat tenderization by the proteolytic enzymes.

4. Conclusion

From the results obtained in this experiment, it was proved that the treatment of tough meat, after osmotic dehydration, was effective in tenerizing it.

Acknowledgements

We wish to express our thanks to Drs. T. Fukuyama, S. Niimura, and T. Sugiyama, University of Niigata, for their kind advice regarding the various electronmicroscopic observations employed in this study.

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