Asian-Aust. J. Anim. Sci.
Vol. 20, No. 6 : 994 - 1001
June 2007

www.ajas.info

Combined Effects of High Pressure and Heat on Shear Value and Histological Characteristics of Bovine Skeletal Muscle

H. Rusman, B. Gerelt[1], S. Yamamoto[2], T. Nishiumi[2] and A. Suzuki[2,]*

Department of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia

ABSTRACT : Changes in shear force value, transverse sections, myofibrils and intramuscular connective tissue of bovine skeletal muscle exposed to the combination of high-pressure up to 400 MPa and heat (30 and 60°C) were studied. The shear force value decreased by pressure-heat treatment up to 200 MPa at 30 and 60°C, and then slightly increased over 200 MPa at 30°C. Shear force values of treated muscles were lower than those of untreated ones. Gaps between muscle fibers in the untreated muscle were a little clear, and then they became very clear in the treated muscles up to 200 MPa at 30 and 60°C. However, the gaps reduced significantly over 200 MPa at 30°C. The remarkable rupture of I-band and loss of M-line materials progressed in the myofibrils with increasing pressure applied. However, degradation and loss of the Z-line in myofibrils observed in the muscle treated at 60°C was not apparent in the muscle treated at 30°C. The length of the sarcomere initially contracted by pressure-heat treatment of 100 MPa at 30°C seemed to have recovered with increase of the pressure up to 400 MPa. In the muscle treated at 60°C, the length of sarcomere gradually decreased with increase of the pressure up to 400 MPa. In the treated muscles, changes in the honeycomb-like structure of endomysium were observed and accelerated with increase of the pressure. A wavy appearance clearly observed at the inside surface of endomysium in the untreated muscles gradually decreased in the treated muscles with increase of the pressure. Tearing of the membrane was observed in the muscles treated over 150 MPa at 30°C, as observed in the sample pressurized at 100 MPa at 60°C. The roughening, disruption and fraying of the membrane were observed over 200 MPa at 60°C. From the results obtained, the combination of high-pressure and heat treatments seems to be effective to tenderize tough meat. The shear force value may have some relationship with deformation of intramuscular connective tissue and myofibrils. (**Key Words** : High Pressure, Shear Value, Myofibrils, Connective Tissue, Bovine Skeletal Muscle)

INTRODUCTION

Meat tenderness has been resolved into at least two different components, "actomyosin toughness" and "background toughness" (Locker, 1960; Marsh and Leet, 1966; Beilken et al., 1990). Actomyosin toughness is the toughness attributed to the myofibrillar proteins, while the background toughness is due to the connective tissue and other stromal proteins.

Tenderness is one of the most important attributes of meat from consumer's point of view. There are several artificial means of tenderizing meat including physical and chemical methods. High pressure is one of the new techniques for tenderizing meat. Several studies have been documented on tenderization of meat due to structural changes of myofibrils caused by high pressure (Bouton, et al., 1977; Macfarlane and Morton, 1978; Kennick et al., 1980; Locker and Wild, 1984; Suzuki et al., 1990; Suzuki et al., 1992; Suzuki et al., 1996; Cheftel and Culioli, 1997; Jung et al., 2000). However, a little work has been reported on the effects of pressurization on the connective tissue.

In general, at ambient or lower temperature and low pressures (100 MPa), there was little or no structural change (Suzuki et al., 1992; Ueno et al., 1999; Jung et al., 2000). At these respective temperatures, increasing the pressure caused M-line loss, disruption of thin filament organization, thickening of Z-line (Suzuki et al., 1992; Jung et al., 2000), and deformation of endomysium organization (Ueno et al., 1999). However, it is not certain whether the pressure-induced structural changes in the intramuscular connective

* Corresponding Author: A. Suzuki. Tel: +81-25-262-6693, Fax: +81-25-262-6854, E-mail: atsuzuki@agr.niigata-u.ac.jp

[1] College of Food Science and Engineering, Inner Mongolia Agriculture University, Huhehaote 010018, Inner Mongolia, China.

[2] Department of Applied Biological Chemistry, Faculty of Agriculture, Niigata University, Niigata-shi, 950-2181, Japan.

Received January 6, 2006; Accepted January 5, 2007

tissue may significantly affect meat tenderness, due to the lack of measurement of shear force value. Suzuki et al. (1993) reported that no significant differences in the ultrastructure, electrophoretic pattern, thermal solubility and thermograms of the intramuscular collagen isolated from untreated (control) and pressurized muscles were observed.

The finding that the combination of low pressure (up to 150 MPa) and heat treatment (40-80°C) improved tenderness was reported by many workers (Bouton et al., 1977; Macfarlane and Morton, 1978; Locker and Wild, 1984). They suggested that the treatment operated on the myofibrillar component of toughness with a little or no effect on that of the connective tissue. This view has been supported in subsequent studies (Ratcliff et al., 1977; Bouton et al., 1978; Bouton et al., 1982; Beilken et al., 1990). Recently, Ma and Ledward (2004) reported that pressures of 200 MPa at 60 and 70°C for 20 min caused larger and significant decreases in hardness of beef than those of the higher pressures up to 800 MPa.

As mentioned above, the combination of higher pressure up to 400 MPa and heat treatment at 30 and 60°C may be effective to eliminate the background toughness than that of low pressure-heat treatment up to 150 MPa or pressure alone. Although high pressure-heat treatment induced visible modification of the color of raw meat, the color difference was greatly reduced after cooking (Cheftel and Culioli, 1997). Therefore the combination of high pressure and heat may effective to eliminate the toughness of meat for meat processing rather than that for fresh meat. This paper describes the shear force value and histological structures of transverse section, myofibrils and intramuscular connective tissue of bovine skeletal muscle exposed to combination of pressures up to 400 MPa and heat treatments at 30 and 60°C for 5 min.

MATERIALS AND METHODS

Meat

Lean meat was excised from the shoulder of a beef carcass, 2 days after slaughter and stored at -20°C. It was tempered overnight in a cold room (3-4°C) as required and then cut into small pieces (about 5×5×3 cm).

High pressure treatment

Each piece of muscle was vacuum-sealed in a polyethylene bag and pressurized at 100, 150, 200, 300 or 400 MPa for 5 min at about 30 and 60°C, by using a cold isostatic press apparatus NBIP 45-120-70 (Nikkiso KK, Tokyo), according to the previously described procedure (Suzuki et al., 1990).

Measurement of shear force value

Five rectangular shaped samples (1×1×5 cm) were removed from the untreated and treated muscles at all pressure grades that were carried out at 30 and 60°C. Each sample was sheared perpendicular to the direction of muscle fiber with a rectangular shaped shear blade on a Rheometer (Fudoh NRM-2002J, Tokyo). The shear force value for a particular pressure-heat treatment represented average of 15 shear force values.

Scanning electron microscopic (SEM) observation of traverse section

Untreated and treated samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for one day at room temperature. Small pieces of samples (5 mm square) were prepared and washed in distilled water, then put in 1% tannic acid for three hours and washed in distilled water for several hours. Later, these small pieces were post-fixed in 1% osmium tetraoxide overnight in a cold room and were dehydrated through graded ethanol and dried by the *t*-butyl alcohol freeze-drying method (Inoue and Osatake, 1988). The dried specimens were coated with gold and examined using a SEM ABT-55 (Akashi Beam Technology) with accelerating voltage 15 Kv. Micrographs of the specimens were taken at magnification of ×750. The width of the gaps between muscle fibers and endomysial tubes was measured at 50 points of gaps from photograph using a pair of calipers. Fifty points on the photograph were selected at random.

Transmission electron microscopic (TEM) observation of myofibrils

TEM observation of the myofibrils was carried out by the method of Suzuki et al. (1978). Small pieces prepared from untreated and treated muscles were fixed with 3% glutaraldehyde and 1% osmium tetraxide, and embedded in Epon mixture. Thin sections of samples were stained with uranyl acetate-lead citrate. Specimens were examined with electron microscope EM 208S (Philips, Holland) with accelerating voltage 100 kV. The length of sarcomere was measured at 20 sarcomeres from electron micrographs using a pair of calipers.

Scanning electron microscopic (SEM) observation of intramuscular connective tissue

Specimens for SEM were prepared by cell-maceration method of Ohtani et al. (1988). Small pieces of the untreated and treated samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for one day. These pieces were immersed in 10% NaOH for five days; the NaOH solution being replaced every day by a fresh one and then rinsed in distilled water for five days at room temperature. Later, these pieces were put in 1% tannic acid for 3 h, washed in distilled water for several hours and post-fixed in 1% osmium tetraoxide overnight in a cold

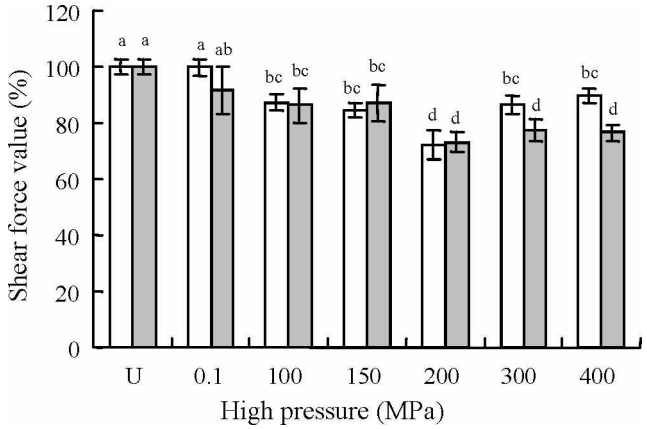

Figure 1. Effect of pressure-heat treatment on shear force value of bovine skeletal muscle. Shear force value was expressed as the percentage of that of the untreated muscle. [a-d] Means with different superscript are significantly different from each other ($p<0.01$). U: untreated; □, Pressure-heated at 30°C; ■, Pressure-heated at 60°C.

room (3-4°C). The specimens were dehydrated through graded ethanol, freeze-fractured in liquid nitrogen and dried by the *t*- butyl alcohol freeze-drying method (Inoue and Osatake, 1988). The dried specimens were coated with gold and examined using a SEM ABT-55 with accelerating voltage 15 kV. Micrographs of the specimens were taken at magnification of ×500.

Statistical analysis

Analysis of variance from Randomized Complete Block Design (RCBD), 6 pressures (untreated = 0.1, 100, 150, 200, 300 and 400 MPa) or 4 pressures (untreated = 0.1, 100, 200 and 400 MPa)×2 temperatures (30 and 60°C), was used to analyze data on width of gaps between muscle, length of sarcomere of muscle and shear force value of muscle. When a significant difference by F-test was observed, we carried out a *t*-test to compare the means (Meyer, 1993).

RESULTS AND DISCUSSION

Effect on shear force

Changes in the shear force values of muscles treated at 30 and 60°C are shown in Figure 1. When pressure treatment was carried out at 30°C, the shear force value of muscles decreased up to 200 MPa and then slightly increased at 300 MPa and 400 MPa (Figure 1). However, the shear force values of treated muscles were lower than those of the untreated ones. The shear force value of muscles treated at 200 MPa was the lowest among others. In the muscle treated at 60°C, the shear forces value decreased by high-pressure and heat treatment up to 200 MPa and retained almost the same value up to 400 MPa (Figure 1).

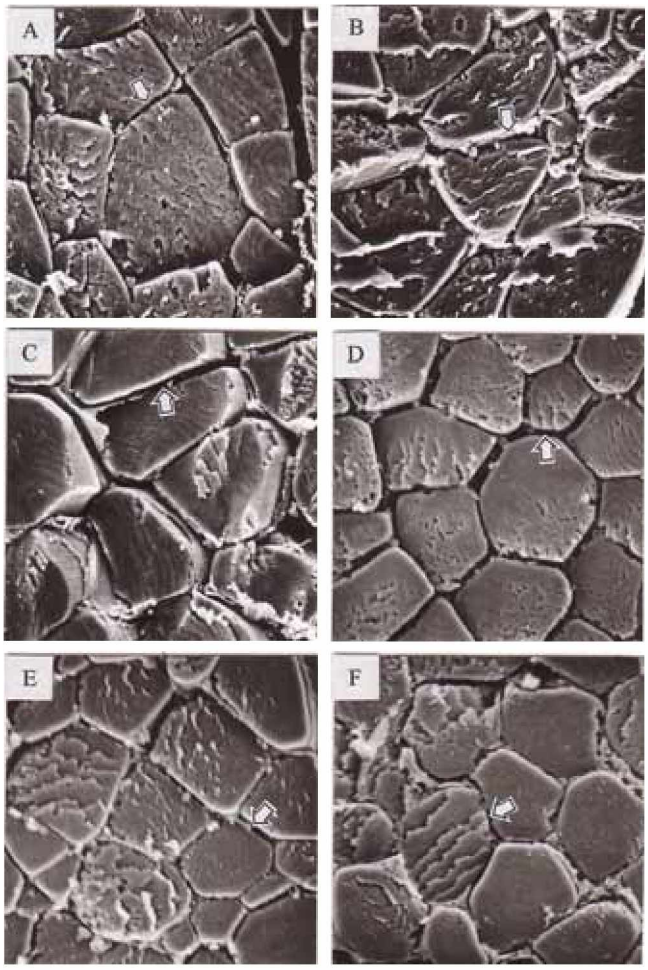

Figure 2. Scanning electron micrograph of transverse sections prepared from bovine skeletal muscles pressure-heated at 30°C. Each scale mark on the electron micrograph indicates 50 μm. Arrows indicate a gap of transverse sections. (A) Untreated; (B) Pressure-heated at 100 MPa; (C) Pressure-heated at 150 MPa; (D) Pressure-heated at 200 MPa, (E) Pressure-heated at 300 MPa; (F) Pressure-heated at 400 MPa, (×750).

The responses of shear force values on pressure-heat treatment at 30°C up to 200 MPa were similar with pressure-heat treatment at 60°C. These results support the previous studies by Bouton et al. (1977) who found no differences on meat tenderness by pressure-heat treatments up to 150 MPa at 35 and 60°C. These results indicate that pressure-heat treatment up to 200 MPa operates on the myofibrillar component of toughness with a little or no effect on that of the connective tissue toughness, because the myofibrillar protein is more sensitive to pressure than that of connective tissue. The similar results have been reported in subsequent studies that used pressure up to 150 MPa (Ratcliff et al., 1977; Bouton et al., 1978; 1982; Beilken et al., 1990).

In contrast, the different results of shear force value were observed between the muscles treated over 200 MPa

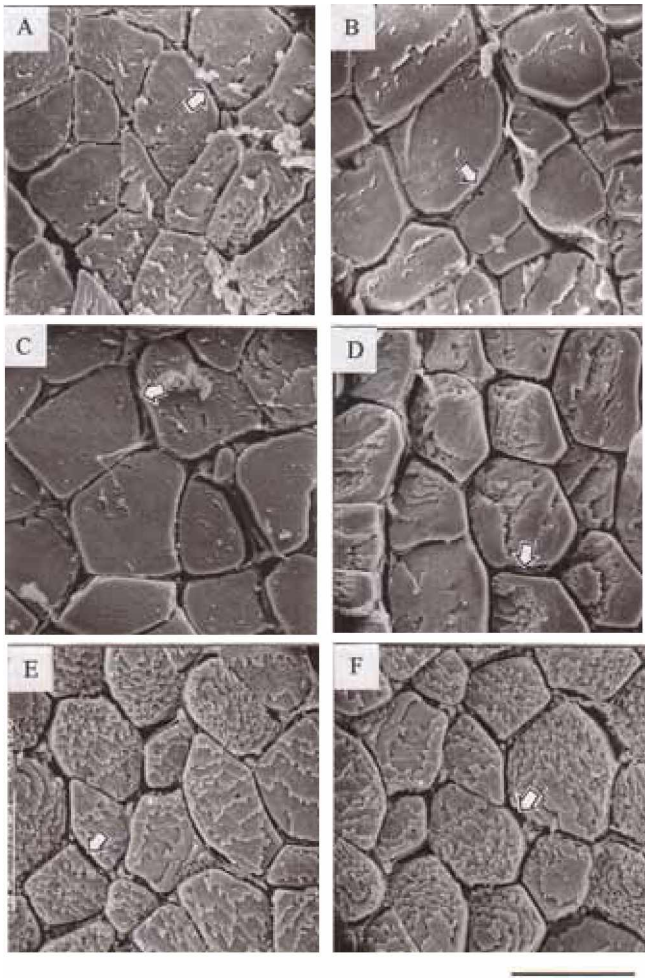

Figure 3. Scanning electron micrograph of transverse sections prepared from bovine skeletal muscles pressure-heated at 60°C. Each scale mark on the electron micrograph indicates 50 μm. Arrows indicate a gap of transverse sections. (A) Untreated; (B) Pressure-heated at 100 MPa; (C) Pressure-heated at 150 MPa; (D) Pressure-heated at 200 MPa; (E) Pressure-heated at 300 MPa; (F) Pressure-heated at 400 MPa, (×750).

at 30 and 60°C. This result may indicate that the combination of high pressure over 200 MPa and heat at 60°C affected on weakening of connective tissue (see Figure 7). Bouton et al. (1981) and Christensen et al. (2000) reported that the decrease in shear force values between 50 and 60°C has been attributed to a weakening of connective tissue. From the results shown in this paper, it appears that application of pressure treatment over 200 MPa at high temperature (60°C) was more effective to resolve "actomyosin" and "background" toughness than that of at low temperature (30°C).

Scanning electron micrograph (SEM) of transverse section

Changes in transverse section of muscles treated at 30 and 60°C are shown in Figure 2 and 3, respectively.

Table 1. Effect of pressure and temperature on the change in width of gaps (μm) between muscle fibers

Pressure	Temperature	
	30°C	60°C
Untreated	2.05±0.24[b]	1.99±0.22[b]
100 MPa	3.72±0.68[a]	3.70±0.59[a]
150 MPa	3.94±0.66[a]	3.74±0.61[a]
200 MPa	4.01±0.47[a]	4.05±0.48[a]
300 MPa	1.86±0.28[b]	3.73±0.54[a]
400 MPa	1.89±0.27[b]	3.90±0.50[a]

[a,b] Means in the same column by different letters are significantly different ($p<0.01$).

As shown in Figure 2, there were no changes in cross surface of muscle fibers, except the gap between muscle fibers. Gaps between muscle fibers and endomysial tubes were a little clear in the untreated muscle. The gaps became very clear on the treated muscles at 100, 150 and 200 MPa. However, with increasing pressure to 300 and 400 MPa, these gaps became much closer (Figure 2). When the pressure treatment was carried out at 60°C (Figure 3), damage in the cross surface of muscle fiber, especially at 300 and 400 MPa, probably due to the slight fragmentation of myofibrils (Figure 5D) were observed. The gaps between muscle fibers and endomysial tubes of all of treated muscles were very explicit. Changes in the width of gaps between muscle fibers of untreated and treated muscles were summarized in Table 1. When pressure treatment was carried out at 30°C, width of gaps of muscles treated at 100, 150 and 200 MPa were greater ($p<0.01$) than those of untreated and muscles treated at 300 and 400 MPa. At 60°C, the width of gaps of all treated muscles was significantly wider ($p<0.01$) than those of untreated ones. However no significant differences among muscles treated from 100-400 MPa at 60°C were observed.

In the gap, there are not only endomysium networks, but may be also water-soluble proteins present, because of a leakage of water-soluble proteins from cells caused by pressure-heat treatment. The combination of pressure and heat over 200 MPa at 30 and 60°C may induce the different effect on the transverse section of muscle fibers. Offer et al. (1984) and Palka and Daun (1999) suggested that shrinkage of muscle fibers of meat during cooking about 45-60°C was primarily transverse. The gap was probably affected by status of sarcomere contraction. We also observed that the length of the sarcomere initially contracted by pressure-heat treatment at 100 MPa at 30°C seemed to have recovered with the increase of the pressure up to 400 MPa, because of the increasing loss of structural continuity. In the muscle treated at 60°C, the length of sarcomere gradually decreased with the increase of pressure up to 400 MPa (Figures 4 and 5). Thus, the difference in the width of gaps observed between muscles pressure-heated at 30°C and 60°C may be

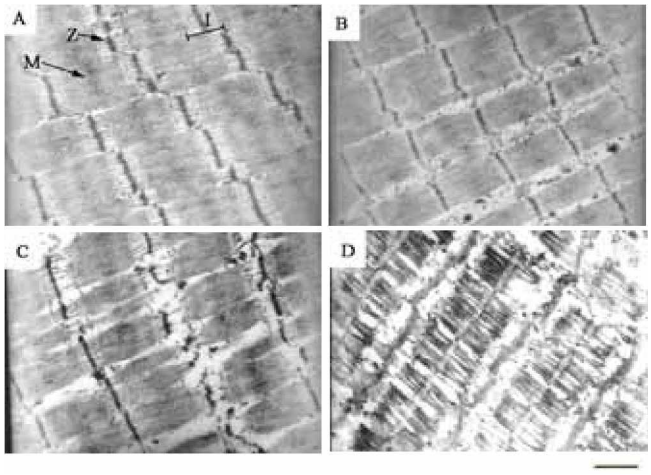

Figure 4. Transmission electron micrograph of myofibril prepared from bovine skeletal muscles pressure-heated at 30°C. Each scale mark on the electron micrograph indicates 1 μm. Scale mark between Z-lines indicates the length of sarcomere. (I) I-band, (M) M-line; (Z) Z-line; (A) untreated; (B) Pressure-heated at 100 MPa; (C) Pressure-heated at 200 MPa; (D) Pressure-heated at 400 MPa, (×15,000).

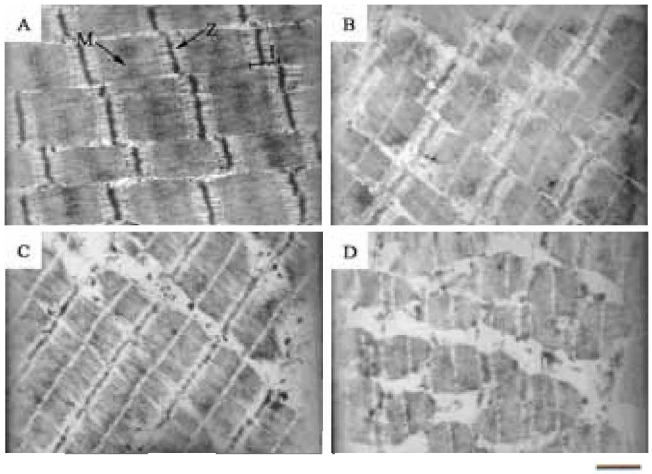

Figure 5. Transmission electron micrograph of myofibril prepared from bovine skeletal muscles pressure-heated at 60°C. Each scale mark on the electron micrograph indicates 1 μm. Scale mark between Z-lines indicates the length of sarcomere. (I) I-band; (M) M-line; (Z) Z-line; (A) untreated; (B) Pressure-heated at 100 MPa; (C) Pressure-heated at 200 MPa; (D) Pressure-heated at 400 MPa, (×15,000).

affected by the difference in the degree of the coagulation density of proteins, contraction of sarcomere and in the status of the endomysium caused by the pressure-heat treatment.

The changes in the gaps between muscle fibers and endomysial tubes probably affected the shear force value. In the muscles treated at 30°C, rehardening of meat followed recovering of gap over 200 MPa. While, in the muscle treated at 60°C, the gaps retained over 200 MPa gave no significant changes in the shear force value.

Table 2. Effect of pressure and temperature on the change in length of sarcomere (μm) of muscles

Pressure	Temperature	
	30°C	60°C
Untreated	2.29±0.044[b]	2.28±0.066[a]
100 MPa	1.79±0.068[c]	2.10±0.071[b]
200 MPa	2.35±0.193[ab]	1.75±0.068[c]
400 MPa	2.48±0.088[a]	1.40±0.075[d]

[a, b, c, d] Means in the same column by a different letter are significantly different ($p<0.01$).

Transmission electron micrograph (TEM) of myofibrils

Transmission electron micrographs of the myofibrils of muscles treated at 30 and 60°C are shown in Figure 4 and 5, respectively. When pressure treatment was carried out at 30°C (Figure 4), the ultrastructure of myofibrils of treated muscle was clearer than that of muscle pressure-heated at 60°C (Figure 5). In the muscle treated at 100 MPa, no remarkable change in the ultrastructure of myofibrils was observed as compared with that of the untreated muscle, except for contraction of sarcomere. The slight loss of a defined filamentous structure through the sarcomere and the deformation of Z-line due to the disruption of I-filament were observed in the muscle treated at 200 MPa. In the muscle treated at 400 MPa, complete loss of the M-line and thickening of the Z-line were observed, which probably due to the collapse of the I-filament. Cleavage of the A-band adding to the many changes already mentioned was observed in myofibrils. It seems that, over 200 MPa, the deformation of myofibrils would not cause tenderness (Figure 1). This result is also in agreement with reports that in the meat treated with 300 MPa at low temperature, the tenderness did not seem to be improved, although the treatment caused ultrastructural modification (Jung et al., 2000). As shown in Figure 5, a slight loss of M-line structure and a little contraction of sarcomere were observed in the muscle treated at 100 MPa. These changes were accelerated with the increase of the pressure-heat treatment up to 400 MPa. In the myofibrils prepared from the muscle treated at 200 MPa, the structural continuity of the sarcomere was slightly lost, with the collapse of I-filament and of the Z line in several places. Acceleration of these changes may affect the slight fragmentation of myofibril on muscle treated at 400 MPa.

The length of the sarcomere initially contracted by pressure-heat treatment at 100 MPa at 30°C (Figure 4B) seemed to have recovered with the increase of the pressure up to 400 MPa (Figure 4C, 4D), because of the increasing loss of structural continuity. In the muscle treated at 60°C, the length of sarcomere gradually decreased with the increase of pressure up to 400 MPa. Changes in the length of sarcomeres of untreated and treated muscles were summarized in Table 2.

It has been reported by many workers (Bouton et al.,

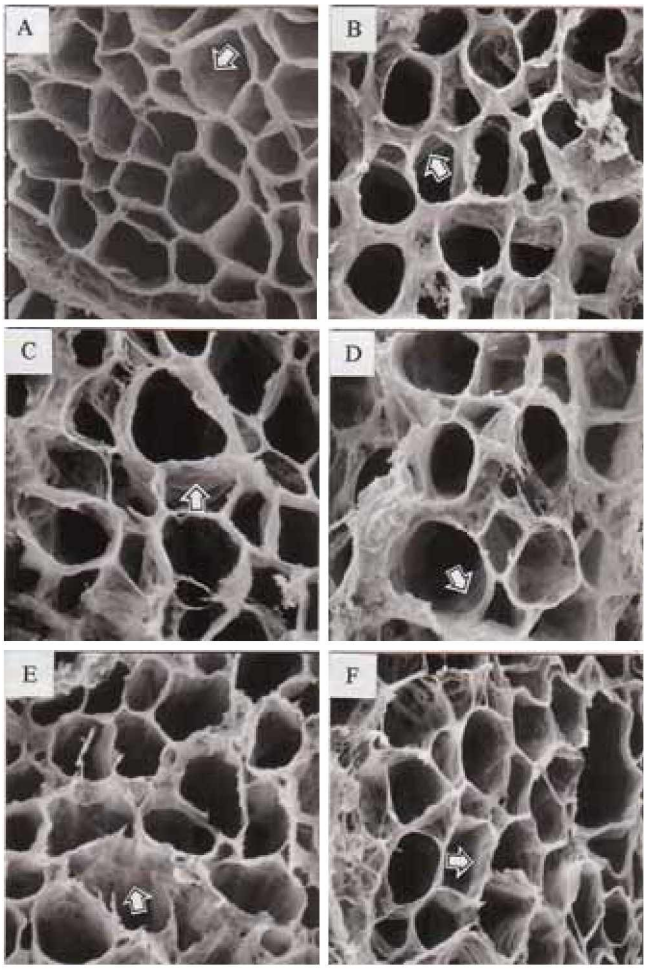

Figure 6. Scanning electron micrograph of intramuscular connective tissue prepared from bovine skeletal muscles pressure-heated at 30°C. Each scale mark on the electron micrograph indicates 50 μm. Arrows indicate wavy appearance of inside surface. (A) Untreated; (B) Pressure-heated at 100 MPa; (C) Pressure-heated at 150 MPa; (D) Pressure-heated at 200 MPa; (E) Pressure-heated at 300 MPa; (F) Pressure-heated at 400 MPa, (× 500).

1977; Macfarlane and Morton, 1978; Kennick et al., 1980; Locker and Wild, 1984; Macfarlane and MacKenzie, 1986; Suzuki et al., 1990; Suzuki et al., 1992; Suzuki et al., 1996; Cheftel and Culioli, 1997) that high pressure treatment of pre- or post-rigor muscle caused a great change in the ultrastructure of myofibril. Several of these workers (Bouton et al., 1977; Macfarland and Morton, 1978; Locker and Wild, 1984) combined the pressure up to 150 MPa with heat treatment to clarify effect on myofibril and connective tissue related with meat tenderness. They suggested that the treatment operated on the myofibrillar component of toughness with a little or no effect on that of the connective tissue. However, in open literature, no worker used pressure-heat treatment over 150 MPa on beef muscle, except Ma and ledward (2004), that described the effects of combined high pressure (200-800 MPa) and heat (20-70°C) on the texture of beef muscle. As the results in Figure 4 and Figure 5, the difference in the pressure-heat induced change was clearly observed in the ultrastructure of myofibril between the pressure-heated at 30°C (Figure 4) and the pressure-heated at 60°C (Figure 5). As shown in Figure 4 and 5, the myofibrils prepared from muscles treated at 30°C (Figure 4B, C, D) was more explicit than that of the muscle treated at 60°C (Figure 5B, C, D), probably due to protein coagulation by combination of pressure and high temperature (60°C). When exposed to treatment at 30°C, the loss of the M-line was observed at muscle treated at 400 MPa, while it was observed at muscles treated at lower pressure at 60°C. In the myofibrils treated at 400 MPa at 30°C, almost completely loss of the structural continuity of sarcomere and the thickening of Z line were observed. However, fragmentation of myofibril was not observed. Otherwise, in the myofibrils treated at 400 MPa at 60°C, the slight fragmentation of myofibrils was observed, due to almost completely loss of I-band and Z-line. These are probably caused by protein coagulation effect of high temperature (60°C) that gradually increased with the increase of pressure. The disruption and deformation of myofibrils may have some relationships with shear force value and the gap of muscle fibers.

Scanning electron micrograph (SEM) of connective tissue

Scanning electron micrographs of the connective tissue of muscles treated at 30 and 60°C are shown in Figure 6 and 7, respectively. In the muscle treated at 30°C, slight structural changes in the endomysium and perimysium, especially deformation of honeycomb-like structure of endomysium were observed and accelerated with the increase of the pressure. A network of wavy collagen fibers was clearly observed on the inside surface of the endomysium in the untreated muscle (arrow in Figures 6A-F and 7A-F). However, it gradually lost in the treated muscle with the increase of the pressure. The development of the smooth surface associated with the disappearance of the wavy structure was already observed in the sample pressurized at 100 MPa (Figures 6B and 7B), probably due to process of stretching of collagen fibers by pressure-heat treatment. Tearing of the endomysium membrane was observed in the muscle treated over 150 MPa (Figure 6C). The slight deformation of endomysium of muscle treated at 30°C probably would not cause the decrease of the background toughness.

Structural changes in the endomysium and perimysium, especially deformation of honeycomb-like structure of endomysium in the muscle treated at 60°C were observed and accelerated with the increase of the pressure (Figure 7).

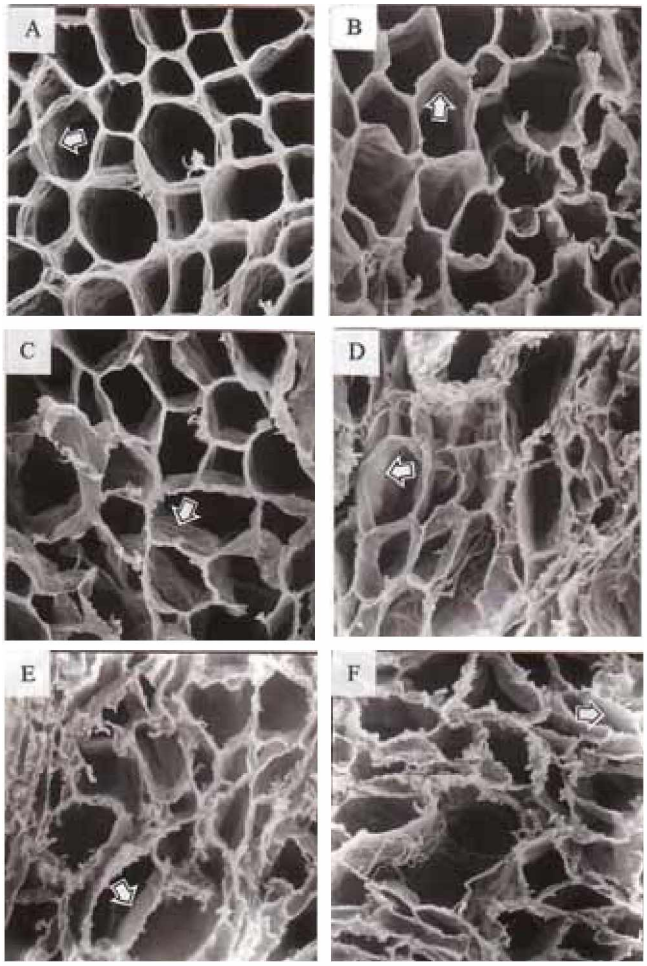

Figure 7. Scanning electron micrograph of intramuscular connective tissue prepared from bovine skeletal muscles pressure-heated at 60°C. Each scale mark on the electron micrograph indicates 50 μm. Arrows indicate wavy appearance of inside surface. (A) Untreated; (B) Pressure-heated at 100 MPa; (C) Pressure-heated at 150 MPa; (D) Pressure-heated at 200 MPa; (E) Pressure-heated at 300 MPa; (F) Pressure-heated at 400 MPa, (×500).

A dense network of wavy collagen fibers was clearly observed on the inside surface of endomysium in the untreated muscle. However, it gradually decreased and disappeared in treated muscles with the increase of the pressure. The endomysium membrane of treated muscles appeared thinner than those of the untreated ones did. Tearing of the endomysium membrane was observed in the muscles treated over 100 MPa, as observed in the sample pressurized at 150 MPa at 30°C (Figure 6). The roughening, disruption and fraying of the endomysium membrane were observed over 200 MPa. Besides, denaturation of proteins may be occurred on muscles treated at 60°C. Therefore, it appears that pressure-heat treatment at 60°C may have a drastic effect on the membrane structure of the intramuscular connective tissue than pressure-heat treatment at 30°C, especially the pressure over 200 MPa. The deformation of connective tissue may have some relationship with the shear force value, gaps between muscle fibers and deformation of myofibrils. The changes in the extractability of proteogycans (PGs) from the muscles treated by high pressure and heat treatments were not determined in this paper. Further study is required to clarify the relationship between the changes in the honeycomb-like structure of endomysium and the extractability of PGs.

CONCLUSION

The pressure-heat treatment up to 200 MPa at 30°C and 60°C may operate on the myofibrillar component of toughness with a little or no effect on that of connective tissue. The contribution of connective tissue to meat toughness may be reduced at pressure over 200 MPa at 60°C. The pressure up to 200 MPa seems to be effective to decrease shear force value at the low temperature (30°C), otherwise at 60°C (high temperature) will be more effective at the pressure from 200 MPa. The shear force values of treated muscles were lower than those of the untreated ones. The application of high pressure-heat treatment causes some deformation of intramuscular connective tissue and myofibrils. The shear force value may have some relationship with the gaps between muscle fibers and deformation of intramuscular connective tissue and myofibrils. From the results obtained, the combination of high-pressure and heat treatments seems to be effective to tenderize tough meat.

ACKNOWLEDGMENT

This work was supported in part by Grant-in-Aid for Scientific Research (A: 15208025) from the Japan Society for the Promotion of Science (JSPS) to A.S.

REFERENCES

Beilken, S. L., J. J. Macfarlane and P. N. Jones. 1990. Effect of high pressure during heat treatment on the Warner Bratzler shear force values of selected beef muscle. J. Food Sci. 55:15-18, 42.

Bouton, P. E., A. L. Ford, P. V. Harris, J. J. Macfarlane and J. M. O'Shea. 1977. Pressure-heat treatment of post rigor muscle. Effect on tenderness. J. Food Sci. 42:132-135.

Bouton, P. E., A. L. Ford, P. V. Harris and F. D. Shaw. 1978. Effect of low voltage stimulation of beef carcasses on muscle tenderness and pH. J. Food Sci. 43:1392-1396.

Bouton, P. E., P. V. Harris and D. Ratcliff. 1981. Effect of cooking temperature and time on the shear properties of meat. J. Food Sci. 40:1122-1128.

Bouton, P. E., A. L. Ford, P. V. Harris, J. J. Macfarlane and W. R.

Shorthose. 1982. Influence of pH on the Warner-Bratzler shear properties of mutton. Meat Sci. 6:27-36.

Cheftel, J. C. and J. Culioli. 1997. Effects of high pressure on meat: a review. Meat Sci. 46:211-236.

Christensen, M., P. P. Purslow and L. M. Larsen. 2000. The effect of cooking temperature on mechanical properties of whole meat, single muscle fibres and perimysial connective tissue. Meat Sci. 55:301-307.

Inoue, T. and H. Osatake. 1988. A new drying method of biological specimens for scanning electron microscopy: The *t*-butyl alcohol freeze-drying method. Arch. Histolo Cytolo. 51:53-59.

Jung, S., M. D. Lamballerie-Anton and M. Ghoul. 2000. Modifications of ultrastructure and myofibrillar proteins of post-rigor beef treated by high pressure. Lebensm.-Wiss.-Technolo. 33:313-319.

Kennick, W. H., E. A. Elgasim, Z. A. Holmes and P. F. Meyer. 1980. The effect of pressurization of pre-rigor muscle on post-rigor meat characteristics. Meat Sci. 40:33-40.

Locker, R. H. 1960. Degree of muscular contraction as a factor in tenderness of beef. Food Res. 25:304-307.

Locker, R. H. and D. J. C. Wild. 1984. Tenderization of meat by pressure-heat involves weakening of the gap filaments in myofibrils. Meat Sci. 10:207-233.

Ma, H-J. and D. A. Ledward. 2004. High pressure/thermal treatment effects on the texture of beef muscle. Meat Sci. 68.347-355.

Macfarlane, J. J. and I. J. Mckenzie. 1986. Pressure-accelerated changes in the protein of muscle and their influence on Warner-Bratzler shear values. J. Food Sci. 51:516-517, 525.

Macfarlane, J. J. and D. J. Morton. 1978. Effects of pressure treatment on the ultrastructure of striated muscle. Meat Sci. 2:281-288.

Marsh, B. B. and N. G. Leet. 1966. Studies in meat tenderness III. The effect of cold shortening on tenderness. J. Food Sci. 31:450-459.

Meyer, G. E. 1993. SPSS A Minimalist Approach. Harcourt Brace Jovanovich College Publishers, Florida, USA.

Offer, G., D. Restall and J. Trinick. 1984. Water-holding capacity in meat. In:Recent Advances in Chemistry of Meat (Ed. A. J. Baily). The Royal Society of Chemistry, pp. 71-86.

Ohtani, O., T. Ushiki, T. Taguchi and A. Kikuta. 1988. Collagen fibrillar networks as skeletal frameworks; a demonstration by the cell maceration/scanning electron microscope method. Arch. Histolo. Cytolo. 51:249-261.

Palka, K. and H. Daun. 1999. Changes in texture, cooking losses, and myofibrillar structure of bovine *M. semitendinosus* during heating. Meat Sci. 51:237-243.

Ratcliff, D., P. E. Bouton, A.L. Ford, P. V. Harris, J. J. Macfarlane and J. M. O'Shea. 1977. Pressure-heat treatment of post-rigor muscle: Objective-subjective measurements. J. Food Sci. 42:857-859, 865.

Suzuki, A., M. Saito, H. Sato and Y. Nonami. 1978. Effects of materials released from myofibrils by CAF (Ca^{2+}-activated factor) on Z-disk reconstitution. Agric. Biolo. Chem. 42:2111-2116.

Suzuki, A., M. Watanabe, K. Iwamura, Y. Ikeuchi and M. Saito. 1990. Effects of high pressure treatment on the ultrastructure and myofibrillar protein of beef skeletal muscle. Agric. Biolo. Chem. 54:3085-3090.

Suzuki A, M. Watanabe, Y. Ikeuchi and M. Saito. 1992. Pressure effects on the texture, ultrastructure and myofibrillar protein of beef skeletal muscle. In: Proceeding of the 38th International Congress Meat Science and Technology. Clermont-Ferrand, France. pp. 423-425.

Suzuki, A., M. Watanabe, Y. Ikeuchi, M. Saito and K. Takahashi. 1993. Effects of high pressure treatment on the ultrastructure and thermal behaviour of beef intramuscular collagen. Meat Sci. 35:17-25.

Suzuki, A., K. Kim and Y. Ikeuchi. 1996. Understanding the pressure effects on postmortem muscle. In: High Pressure Bioscience and Biotechnology (Ed. R. Hayashi and C. Balny). Elsevier Science B.V., Amsterdam. pp. 289-297.

Ueno, Y., Y. Ikeuchi and A. Suzuki. 1999. Effects of high pressure treatments on intramuscular connective tissue. Meat Sci. 52: 143-150.