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Effects of Hydrostatic Pressure Treatment on the Physicochemical, Morphological, and Textural Properties of Bovine *Semitendinosus* Muscle

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Abstract The effects of hydrostatic pressure (HP) treatment on the physicochemical, morphological, and textural properties of bovine *semitendinosus* (ST) muscle were assessed. Based on SDS-PAGE, the decrease in HP-treated ST muscle protein solubility in 0.1 M KCl buffer (pH 7.0) was attributable to a reduction in the levels of sarcoplasmic protein, and the protein solubility decrease observed in 0.6 M KCl buffer (pH 7.0) was attributable to a reduction in the levels of myosin heavy-chain and actin. Scanning electron microscope (SEM) observations showed that muscle fibers became finer and more compact with increasing pressures. The shear force and hardness of ST muscle pressurized to 300 MPa decreased significantly ($p<0.05$), however samples pressurized at 100 and 500 MPa exhibited a significant increase in both attributes relative to the control sample ($p<0.05$).

Keywords: hydrostatic pressure, bovine *semitendinosus* muscle, shear force, texture profile analysis, scanning electron microscope

Introduction

Connective tissues and myofibrillar protein are the structural components of meat that most profoundly affect its tenderness or toughness. Myofibrillar protein, in particular, may be responsible for much of the toughness of cooked meat, which is seen primarily in muscle that has entered rigor mortis in the contracted state (1). A direct relationship has been reported between the tenderness of meat and the weakening of myofibrils (2). The structural weakening of myofibrils may be caused by the weakening of Z-lines, the weakening of rigor linkages formed between actin and myosin, the splitting of connectin (titin) filaments, and the fragmentation of nebulin filaments (3). Koohmaraie *et al.* (2) reported that tougher meat tends to have larger fibers and shorter sarcomeres.

The application of hydrostatic pressure (HP) to meat can induce structural changes (4, 5), and thus also affects the tenderness or toughness of the meat (6). With regard to structural changes, HP-treated meat exhibits alterations in the A-band, a weakening of the gap filaments, a loss in the integrity and aggregation of the I-band, breaks in the Z-lines, and larger myofibrils (7-11). Comprehensively, these HP-induced changes in meat texture may be dependent on the treatment conditions (pressure level, duration, and temperature) (12), the muscle type, and the stage of rigor mortis.

In this study, the effects of HP treatment on the physicochemical, morphological, and textural properties of *semitendinosus* (ST) muscles were evaluated.

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Materials and Methods

Sample preparation ST muscles were obtained from carcasses (Holstein) 48 hr after slaughter, and then cut parallel to the myofibril axis. The samples were cut into 3 cm-thick, 150 g steaks.

Hydrostatic pressure treatment The equipment used in this study included an isostatic pressure unit (Quintus food processor 6; ABB Autoclave Systems, Inc., Columbus, OH, USA). Forty eight hr post-mortem, the ST muscles were positioned inside a high pressure vessel submerged in a hydrostatic fluid medium. The samples were treated at pressures of 100, 200, 300, 400, and 500 MPa for 5 min, with the initial temperature of the pressure vessel at 15± 3°C. Following treatment, all of the samples were stored at 4°C until required.

Measurement of protein solubility Protein solubility was determined in accordance with the procedure described by Li-Chen (13). Meat homogenates in 0.1 M KCl buffer (pH 7.0) were centrifuged at 10,000×g for 30 min at 4°C, and the protein concentration of each supernatant was determined via the Biuret method (14). The supernatant was analyzed for protein as described above, and compared with total protein content in accordance with the following equation:

$$\text{Protein solubility (\%)} = (\text{protein}_{\text{supernatant}}/\text{protein}_{\text{uncentrifuged sample}}) \times 100$$

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) SDS-PAGE was conducted on 12.5% polyacrylamide gels containing 1% SDS (15). The electrophoresis sample was dissolved in Tris-HCl buffer (pH 7.5) containing 8 M urea, 2% SDS, and 2% 2-

mercaptoethanol, then heated for 23 min at 100°C. Fixation and Coomassie brillant blue staining were conducted as described by Neuhoff *et al.* (16).

pH, meat color, and water holding capacity measurements The pH of each sample was determined using a pH meter (pH meter 430; Corning, New York, NY, USA) with a combined glass electrode in suspensions containing 2 g of sample in 20 mL of distilled water following 1 min of mixing. The color of the meat was measured with a Minolta colorimeter (Chroma Meter CR-200; Minolta Camera Co., Osaka, Japan), with the CIE color scale (lightness, L^*; redness, a^*; yellowness, b^*). The water holding capacity (WHC) was assessed using a centrifugal method (17). The meat sample was placed on a polyester membrane (pore size 5 μm), which was maintained in the center of a centrifuge tube by means of plastic beads. After 5 min of centrifugation at 120×g, the WHC was expressed as a percentage of the initial water content of the gel (18).

Measurement of cooking loss Each HP-treated meat sample held in a plastic bag, and immersed in a water bath at 75°C, until the internal temperature reached 75°C. The bag was then cooled for 30 min under running tap water. The juice was drained off and the meat gently blotted dry with paper towels, and reweighed. Cooking loss was determined by mass difference, and expressed as the percentage of loss relative to the initial weight.

Measurement of Ca^{2+}- and Mg^{2+}-ATPase activities Ca^{2+}- and Mg^{2+}-ATPase activities were determined in accordance with the methods of Jiang *et al.* (19) with minor modification. The Ca^{2+}-ATPase activity of myofibrils from HP-treated meat samples was measured in a reaction mixture containing 5 mM $CaCl_2$, 100 mM KCl, 25 mM Tris-maleate (pH 7.0), 1 mM ATP, with 5 mg/mL protein at 25°C. Similarly, Mg^{2+}-ATPase activity was measured in 2 mM $MgCl_2$, 100 mM KCl, 25 mM Tris-maleate (pH 7.0), 1 mM ATP, with 5 mg/mL protein at 25°C. The ATPase reaction was initiated with the addition of ATP, and terminated with the addition of 15% perchloric acid. The amount of liberated inorganic phosphate was determined in accordance with the method developed by Fiske and Subbarow (20).

Scanning electron microscopy (SEM) The pressurized samples were cut into pieces approximately 2 mm^3 with a sharp clean blade. Pieces were fixed in 2.5% glutaraldehyde in sodium phosphate buffer pH 7.0 for 2 hr at 4°C, then rinsed in 0.2 M sodium phosphate buffer at pH 7.0. After washing, the samples were post-fixed in 1% osmium tetroxide for 1 hr at 4°C, and dehydrated in 25, 50, 70, 98%, and absolute ethanol for 20 min per solution (21-23).

Texture measurements Warner-Bratzler (WB) shear force was determined using 10-12 sub-samples, each with a 1 cm-diameter cross-section and a length of 3-4 cm, with the fibers set perpendicular to the direction of a blade attached to a texture analyzer (TA-XT2; Stable Micro Systems Ltd., Godalming, Surrey, UK).

Texture profile analysis (TPA) was conducted with a TA-XT2. The samples were compressed to 50% of their original height using a cylindrical piston with a 50 mm diameter. The TPA parameters were determined as described by Bourne (24, 25).

Statistical analysis The data were analyzed via ANOVA, using the SAS statistical program, and significant differences among the various treatments were compared using Duncan's multiple range tests (26).

Results and Discussion

Protein solubility Figure 1 shows the protein solubility of hydrostatic pressure (HP)-treated bovine *semitendinosus* (ST) muscle samples in 0.1 and 0.6 M KCl buffer (pH 7.0). The protein solubility of ST muscle pressurized to 100 MPa in 0.1 M KCl buffer (pH 7.0) (32.01±1.57%) was not significantly different from that of the control (32.55±1.58%), however solubility decreased gradually with increasing pressure. The percentages of soluble protein from HP-treated ST muscle in 0.1 M KCl buffer (pH 7.0) were 28.87±1.77, 28.50±1.98, 22.31±1.33, and 19.61±0.98% at 200, 300, 400, and 500 MPa, respectively.

The protein solubility of ST muscle pressurized to 100 MPa in 0.1 M KCl buffer (pH 7.0) (54.40±1.23%) was not significantly different from that of the control (54.90±1.02 %), however the solubility decreased gradually with increasing pressure up to 400 MPa. The percentages of soluble protein from HP-treated ST muscle in 0.6 M KCl buffer (pH 7.0) were 47.33±1.01, 41.57±0.58, and 34.87± 0.72% at 200, 300, and 400 MPa, respectively. We detected no significant difference between samples pressurized to 400 and 500 MPa (34.25±1.22%).

SDS-PAGE Changes in the soluble protein content of HP-treated ST muscle in 0.1 and 0.6 M KCl buffer (pH 7.0) were detected via SDS-PAGE (Fig. 2a, 2b). Of the proteins soluble in 0.1 M KCl buffer (pH 7.0) (Fig. 2a),

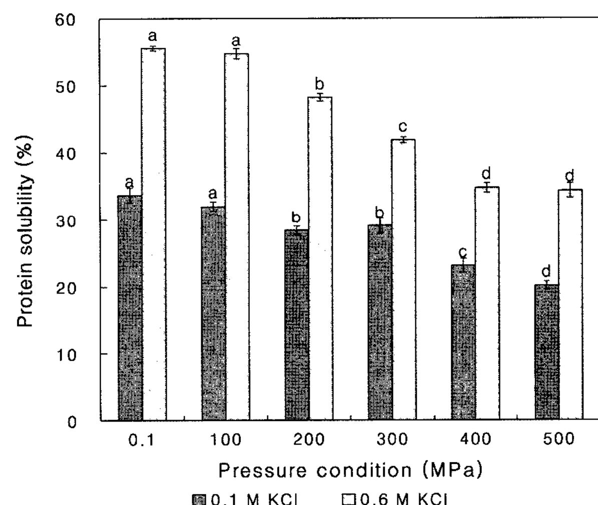

Fig. 1. Changes in the solubility of HP-treated bovine *semitendinosus* muscle protein in 0.1 and 0.6 M KCl buffer (pH 7.0). Bars represent the standard deviation of three determinations. Letters indicate significant differences among groups with same symbol at $p<0.05$ by Duncan's multiple range test.

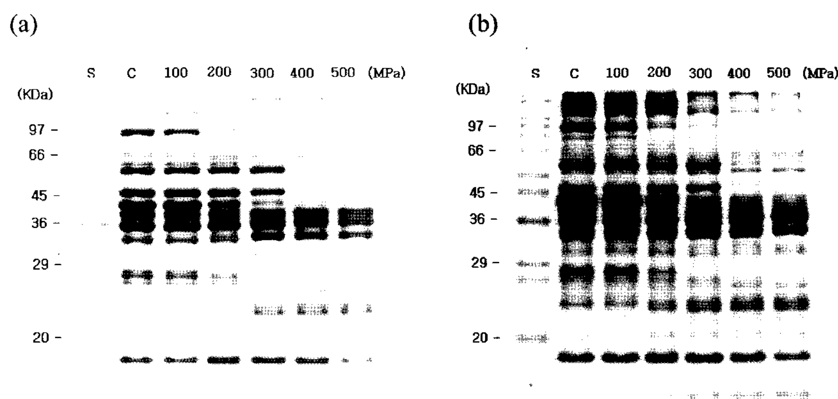

Fig. 2. SDS-PAGE pattern of soluble protein from HP-treated bovine *semitendinosus* muscle in 0.1 (a) and 0.6 M (b) KCl buffer (pH 7.0). Lane S: protein standard with molecular weights indicated on the left margin; lane C: control; lanes 100, 200, 300, 400, 500. hydrostatic pressure treatment at 100, 200, 300, 400, and 500 MPa for 5 min, respectively.

the amount of high molecular weight proteins (above 116 kDa) decreased with increasing pressure. However, we detected no significant difference between the control and ST muscle pressurized to 100 MPa with regard to the amount of 97 kDa molecular weight proteins, although these proteins were not detected in ST muscle pressurized to 200 MPa. The amounts of 46 and 60 kDa molecular weight proteins decreased in ST muscle when pressurized to more than 400 MPa. Also, the amount of 44 kDa molecular weight proteins noticeably decreased in ST muscle pressurized to more than 300 MPa. The SDS-PAGE pattern of low molecular weight proteins (20-36 kDa) from ST muscle samples pressurized to 100 and 200 MPa was similar to that of the control, whereas a different pattern of low molecular weight proteins was detected in ST muscle pressurized to more than 300 MPa. Regarding the SDS-PAGE pattern of proteins soluble in 0.6 M KCl buffer (pH 7.0) (Fig. 2b), the amount of myosin heavy chain (205 kDa) was noticeably lower in ST muscle pressurized to 200 MPa. The amount of actin (44 kDa) decreased gradually with increasing pressure.

pH and meat color Table 1 shows the pH and the Hunter color values of HP-treated ST muscle samples. The pH of HP-treated ST muscle increased with increasing pressures ($p<0.05$). With regard to the color of HP-treated ST muscle samples, the lightness (L*) increased significantly with increasing pressures ($p<0.05$). The redness (a*) of ST muscle samples pressurized to 100 and 200 MPa was not appreciably different from that observed for the control, however the a* value of ST muscle pressurized to 400 and 500 MPa was significantly lower than that of the control. The yellowness (b*) value in ST muscle pressurized to above 300 MPa was slightly elevated ($p<0.05$).

Water holding capacity and cooking loss Figure 3 shows the changes in water holding capacity (WHC) and cooking loss of HP-treated ST muscle samples. The WHC of the control samples was not significantly different from that of ST muscle pressurized to 100 MPa. The WHC of ST muscle treated at 200 MPa was significantly lower than that of samples treated at 100 MPa, but was not significantly different than samples pressurized to above 200 MPa.

The cooking loss for each of the samples increased gradually with increasing pressures up to 300 MPa ($p<0.05$). We noted no significant differences in cooking loss among ST muscles pressurized to 300, 400, and 500 MPa.

Table 1. Changes of pH and meat color in HP-treated bovine *semitendinosus* muscle[1)]

Parameter / Pressure (MPa)	pH	Meat color		
		L*	a*	b*
Control	5.41±0.05$^{c2)}$	39.90±3.07^{d}	25.15±1.60^{ab}	9.69±1.21^{b}
100	5.42±0.03^{c}	41.91±1.74^{cd}	24.47±0.98^{ab}	9.21±0.83^{b}
200	5.49±0.04^{b}	48.77±2.57^{c}	24.68±1.72^{ab}	9.42±1.10^{b}
300	5.56±0.03^{ab}	53.52±2.73^{ab}	25.54±1.63^{a}	11.87±0.65^{ab}
400	5.59±0.02^{a}	55.64±3.93^{ab}	23.87±2.01^{b}	12.05±0.40^{a}
500	5.62±0.06^{a}	56.53±2.63^{a}	20.62±0.90^{c}	11.88±0.45^{a}

[1)]All values are the mean±SD of 5 determinations.
[2)]Letters in the same column represent significant differences at $p<0.05$ by Duncan's multiple range test.

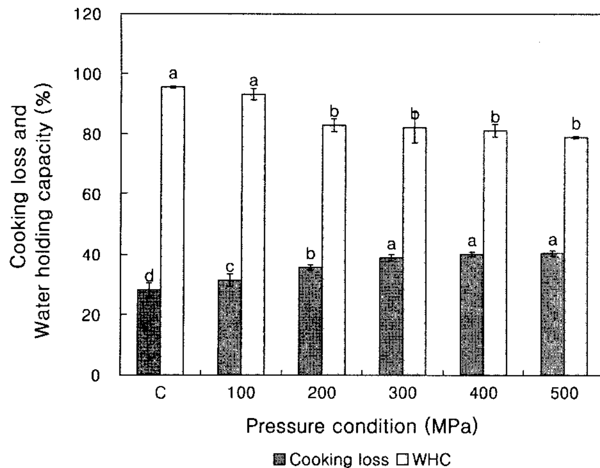

Fig. 3. Changes in cooking loss and water holding capacity (WHC) of HP-treated treated bovine *semitendinosus* muscle. Bars represent the standard deviation from 5 determinations. Letters indicate significant differences among groups with the same symbol at $p<0.05$ by Duncan's multiple range test.

Ca^{2+}- and Mg^{2+}-ATPase activities The Ca^{2+}- and Mg^{2+}-ATPase activities in myofibrils extracted from HP-treated ST muscle samples were also evaluated (Fig. 4). Ca^{2+}-ATPase activity decreased with increasing pressure, and an almost complete loss of Ca^{2+}-ATPase activity occurred at approximately 400 MPa of pressure. However, Mg^{2+}-ATPase activity increased slightly in samples treated at 100 MPa compared to the control, and then decreased substantially at 200 MPa of pressure and above. A complete loss of Mg^{2+}-ATPase activity occurred at approximately 400 MPa.

Scanning electron microscopy (SEM) Changes in the ultrastructure of HP-treated ST muscle were observed via scanning electron microscopy (SEM) (Fig. 5). SEM observations of transverse sections revealed that the muscle fibers became finer and more compact with increasing pressure relative to the control muscles. This may be attributable to the aggregation or coagulation of certain sarcoplasmic proteins or the accumulation of myofibrillar proteins after the breakdown of the muscle fibers (27).

Shear force and texture profile analysis The shear force and texture profile analysis (TPA) of HP-treated ST muscles are shown in Table 2. The texture of HP-treated ST muscles was evaluated after cooking to an internal temperature of 75°C. Changes in the shear force of HP-treated ST muscle were similar to the changes in hardness with increasing pressure. The shear force and hardness of ST muscle pressurized to 100 MPa increased slightly, however a significant reduction ($p<0.05$) in both shear force and hardness was observed in muscle samples pressurized to 300 MPa. The shear force and hardness of ST muscle pressurized to 500 MPa showed a significant increase relative to the control ($p<0.05$). However, no significant differences were observed in samples treated at 200 and 400 MPa relative to the control ($p>0.05$).

The springiness of HP-treated ST muscle samples was

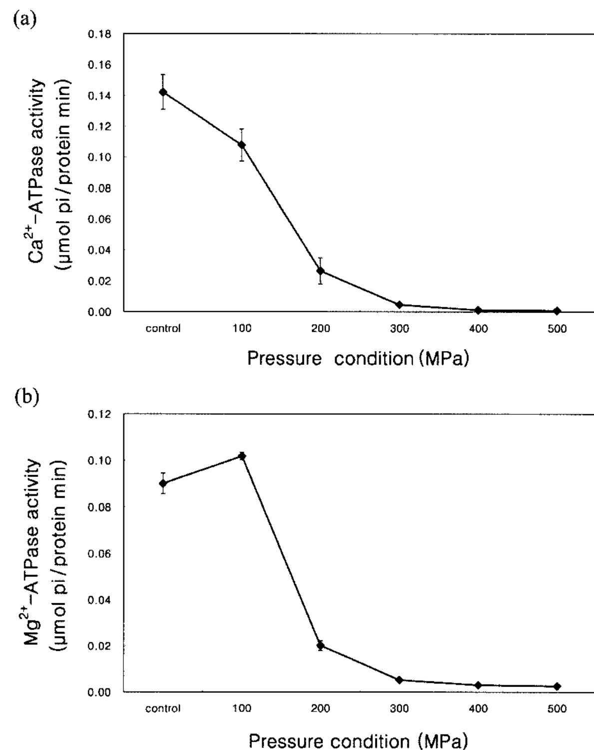

Fig. 4. Changes in Ca^{2+}-ATPase activity (a) and Mg^{2+}-ATPase activity (b) of myofibrillar protein extracted from HP-treated bovine *semitendinosus* muscle in 0.1 M KCl and pH 7.0 (20 mM Tris-HCl buffer). ATPase activity was expressed as moles of Pi liberated from ATP by 1 mg of myofibrils per 1 min.

elevated relative to the control, and this quality reached a maximum value following treatment at 200 MPa. Cohesiveness was significantly different among the groups, and changes in the gumminess of HP-treated ST muscle with increasing pressure were similar to the changes in hardness. The chewiness of HP-treated ST muscles was higher than that of the control, and this quality reached a maximum value following treatment at 500 MPa.

In order to gain further understanding of the effects of HP on the texture of ST muscle, the physicochemical, morphological, and textural properties of ST muscle in relation to pressure were evaluated. According to the results of this study, no definite trends were observed with regard to changes in texture at differing pressures. In other words, each pressure treatment had an idiosyncratic effect on texture. In ST muscle samples pressurized to 100 MPa, the Ca^{2+}-ATPase activity decreased slightly, and the Mg^{2+}-ATPase activity increased relative to the control. These results for ST muscle pressurized to 100 MPa were the same as those for myofibrillar protein suspension pressurized to 100 MPa (28). This may reflect the denaturation of the myosin head, and an increase in the interactions between myosin and actin. These phenomena most likely affected the observed increase in shear force for ST muscle pressurized to 100 MPa. This result is consistent with the findings of MacFarlane *et al.* (9), who reported no difference or only a slight increase in the toughness of meat samples after 3 hr of 150 MPa pressure

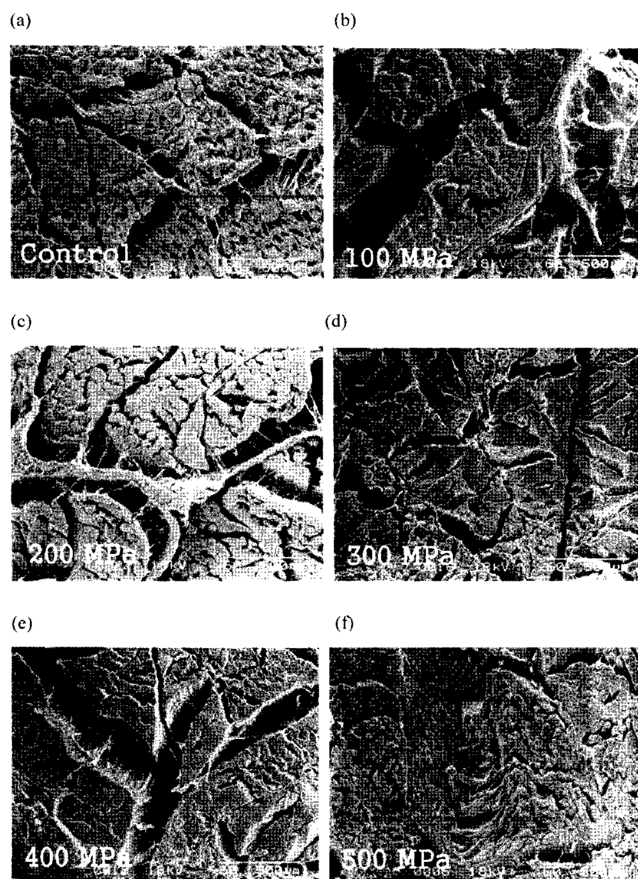

Fig. 5. Scanning electron micrographs of transverse sections from HP-treated bovine *semitendinosus* muscle.

treatment at 0°C.

Based on the Ca^{2+}- and Mg^{2+}- ATPase activities following various pressure treatments, the denaturation of the myosin head and of actin may have progressed, and interactions between myosin and actin may have been weakened in ST muscle pressurized to 200 MPa. Therefore, the shear force of ST muscle pressurized to 200 MPa may be lower than the shear force of ST muscle pressurized to 100 MPa. In ST muscles pressurized to 300 MPa, degradation of the I-band induced by the release of troponin-T and tropomyosin (Fig. 2) may influence the observed reduction in shear force. In ST muscle pressurized to 400 or 500 MPa, the release of troponin-T and tropomyosin was observed in a manner similar to that of ST muscle pressurized to 300 MPa. However, the shear force of these samples was higher than that seen in samples subjected to 300 MPa of pressure. This result is consistent with those reported by Suzuki *et al.* (29), who reported a decrease in the hardness of post-rigor muscle pressurized for 5 min to 300 MPa at 10°C.

In samples subjected to pressures of 400 and 500 MPa, the quantity of soluble proteins between 45 and 66 kDa, which may represent sarcoplasmic protein components, decreased in comparison to other treatments. Therefore, the protein-protein interactions induced by the application of 400 or 500 MPa of pressure might be more stable than those attributable to other treatments and those of the control, and this phenomenon may affect the observed shear force increases. This result is consistent with those reported by Jung *et al.* (11), who reported that HP treatment (520 MPa for 260 sec at 10°C) induced an increase in the catheptic activity of meat, however no conclusive effects were observed with regard to meat tenderization.

Among TPA parameters, changes in the hardness and gumminess of HP-treated ST muscle samples exhibited a trend similar to that seen with changes in the shear force in relation to pressure. The springiness of HP-treated ST muscle increased relative to the control, with a maximum value observed at 200 MPa. Lee *et al.* (30) found that an increase in the moisture content of a frankfurter meat emulsion reduced its springiness. In this study, cooking losses increased gradually with treatment up to 300 MPa, however no differences were observed at pressures above 300 MPa. Changes in cooking losses reflected a gradual decrease in moisture content in the cooked ST muscle subjected to pressures of up to 300 MPa. However, changes in cooking loss did not coincide with changes in springiness. Thus, it may be that moisture content is not the only factor affecting the springiness of HP-treated ST muscle samples. Palka and Daun (31) reported that the springiness of muscle may be related to the degree to which the myofibrillar bundle swells. According to the SEM results (Fig. 5), the muscle fibers became finer and more compact with increasing pressures. Overall, the springiness of ST muscle at pressures above 300 MPa was higher than that of the control sample, but lower than

Table 2. Changes of shear force and texture profile analysis parameters in HP-treated bovine *semitendinosus* muscle[1)]

Pressure (MPa) \ TPA parameter	Shear force (Kg)	Hardness (kg)	Springiness (-)	Cohesiveness (-)	Gumminess (kg)	Chewiness (kg)
Control	$7.45\pm1.50^{b2)}$	2.73 ± 0.29^{bc}	0.75 ± 0.03^{c}	0.60 ± 0.02^{ab}	1.69 ± 0.16^{b}	1.26 ± 0.11^{c}
100	8.17 ± 1.03^{ab}	2.81 ± 0.37^{ab}	0.82 ± 0.02^{b}	0.61 ± 0.02^{a}	1.72 ± 0.24^{ab}	1.41 ± 0.20^{b}
200	7.13 ± 0.78^{b}	2.77 ± 0.13^{bc}	0.86 ± 0.02^{a}	0.59 ± 0.01^{b}	1.67 ± 0.07^{b}	1.52 ± 0.07^{ab}
300	5.91 ± 1.00^{c}	2.59 ± 0.26^{c}	0.84 ± 0.40^{ab}	0.61 ± 0.02^{a}	1.58 ± 0.18^{c}	1.33 ± 0.16^{bc}
400	7.46 ± 1.30^{b}	2.93 ± 0.28^{ab}	0.81 ± 0.04^{b}	0.60 ± 0.02^{ab}	1.74 ± 0.12^{ab}	1.41 ± 0.13^{b}
500	10.02 ± 1.49^{a}	3.31 ± 0.30^{a}	0.80 ± 0.04^{b}	0.60 ± 0.02^{ab}	1.98 ± 0.24^{a}	1.58 ± 0.21^{a}

[1)]All values are the mean±SD of 5 determinations.
[2)]Letters in the same column represent significant differences at $p<0.05$ by Duncan's multiple range test.

samples subjected to pressures of 200 MPa. This is most likely because the moisture content of ST muscle treated at above 300 MPa was lower than the control, and the muscle fibers were more compact than those of samples subjected to 200 MPa of pressure.

Acknowledgments

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