THE EFFECTS OF HIGH-PRESSURE TREATMENT ON INTRAMUSCULAR COLLAGEN

Ichinoseki, S.[1], Nishiumi, T.[2], Sakata, R.[3] and Suzuki, A.[2]

[1] Graduate School of Science and Technology, Niigata University, Niigata 950-2181, Japan – E-Mail: f02d381j@mail.cc.niigata-u.ac.jp
[2] Faculty of Agriculture, Niigata University, Niigata 950-2181, Japan
[3] School of Veterinary Medicine, Azabu University, Sagamihara 229-8501, Japan

Background

Recently, the application of high hydrostatic pressure on food processing was successfully demonstrated as non-heat food processing. High hydrostatic pressurization is also one of the new technologies for tenderizing meat or accelerating meat conditioning (SUZUKI *et al.*, 1998). Meat tenderness was determined by both actomyosin toughness attributed to myofibrillar proteins and background toughness attributed to connective tissue proteins mainly composed of collagen. It has been well known that high-pressure treatment causes the structural and chemical changes in a number of myofibrillar proteins. However, little is known about the effects of pressurization on connective tissue.

Objective

The purpose of this study was to find the effect of high-pressure treatment on the intramuscular collagen characteristics. In this study, the changes of toughness, heat-solubility and the structure of intramuscular collagen on high-pressure treatment were investigated.

Methods

Lean meat was removed from the shoulder of an 86-month-old Holstein cow 1 day after slaughter and stored at –20°C. As required, it was tempered overnight in a cold room at 4°C.

1. *Isolation of intramuscular collagen*

Intramuscular collagen was isolated by the method of FUJII & MUROTA (1982). A part of the isolated collagen was freeze-dried, and remainder was used for the following analysis.

2. *Pressurization*

Muscle or isolated intramuscular collagen was packed in a polyethylene bag, sealed with cold distilled water and pressurized at 100-500 MPa for 5 min at about 8°C using an isostatic press apparatus (Nikkiso KK, Tokyo).

3. *Shear force value*

Muscle samples were cut into small pieces (1x1x2 cm) with the muscle fiber direction along the long axis, and collagen fibers isolated from the muscle were shaped into same size (1x1x2 cm) with plastic case. Shear force value for each sample was measured using a Rheometer (Fudoh NRM-2002J, Tokyo) with a knife-blade.

4. *Heat-solubility of collagen*

The HILL (1966) procedure was used. Each freeze-fractured sample was heated for 70 min at 77°C in distilled water and separated into heat-soluble and insoluble fraction. After hydrolyzing with 6 N HCl for 24 h at 110°C, hydroxyproline concentration of each fraction was determined (BERGMAN & LOXLEY, 1963). Collagen content was calculated by multiplying the hydroxyproline content by 7.25 (insoluble collagen) and 7.52 (soluble collagen). Percentage of soluble collagen was expressed as the heat-solubility of collagen.

5. *Scanning electron microscopy (SEM)*

After pressurization of muscles at 0.1-500 MPa, the samples were fixed in 2.5% glutaraldehyde in phosphate buffer, pH 7.4. The samples cut into small pieces were immersed in 10% NaOH, and then rinsed in distilled water. These pieces were put in 1% tannic acid, rinsed in distilled water, and post-fixed in 1% OsO_4. After dehydration through a graded ethanol, the specimens were freeze-dried by *t*-butyl alcohol. The specimens were coated with gold and observed using a SEM (Hitachi S-430, Tokyo) with an accelerating voltage of 15 kV.

Results and discussions

1. *Shear-force value*

Changes in shear force value during pressurization of muscles and intramuscular collagen fibers were shown in Fig. 1 and Fig. 2, respectively. Shear force value decreased significantly ($p<0.05$) with the pressure applied in both samples. In the previous study, SUZUKI *et al.* (1998) demonstrated that the pressurization, up to 300 MPa, caused meat tenderization, which agreed with our result. As shown in Fig. 2,

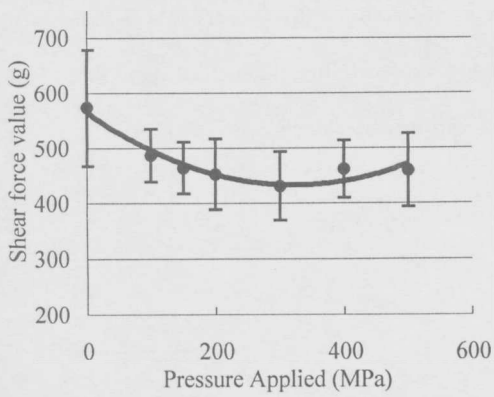

Fig. 1. Effect of high-pressure treatment on shear force value of muscle

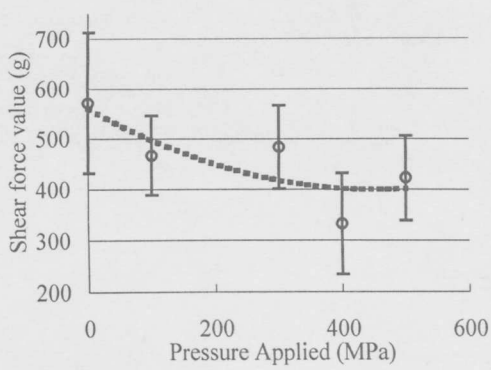

Fig. 2. Effect of high-pressure treatment on shear force value of intramuscular collagen fiber

intramuscular collagen fiber was also tenderized by high-pressure treatment, which suggests that the pressurization may cause some structural and/or chemical changes in the intramuscular collagen.

2. *Heat-solubility of collagen*

It has been demonstrated that a strong correlation exists between heat-solubility of collagen, used as an index of thermal and structural stability of collagen, and meat texture (NISHIUMI *et al.* 1995). In this study, heat-solubility of collagen increased during high-pressure treatment, which indicates a decreasing thermal stability of intramuscular collagen with pressure applied.

3. *SEM observations*

Although UENO *et al.* (1999) found a structural weakening of endomysium in pressurized muscle, we could not observe it in this study. However, perimysium was affected by high-pressure treatment (Fig. 3). Appearance and enlargement of a gap in the region of perimysial-endomysial junction and disintegration of perimysial sheets were observed.

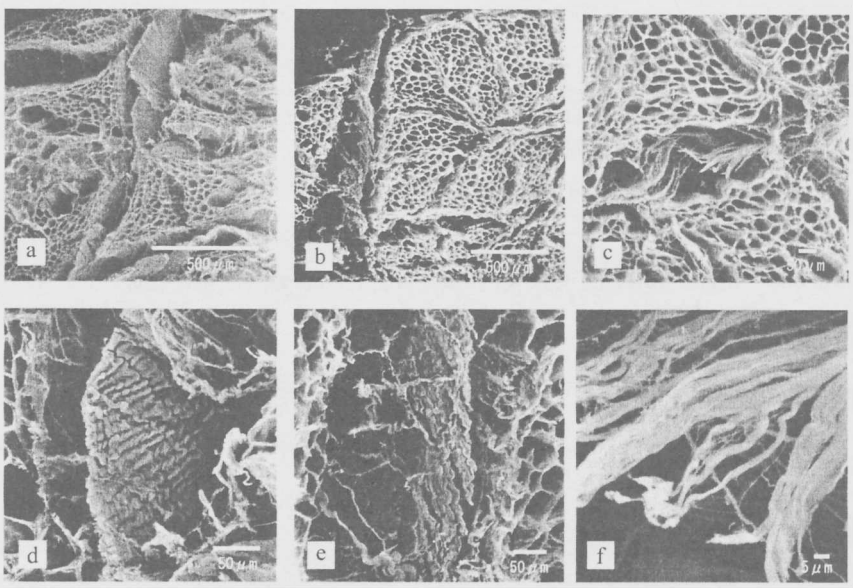

Fig. 3. Scanning electron micrographs of perimysium prepared from the pressurized bovine muscles;
(a) untreated (control), (b) pressurized at 150 MPa, (c) 500 MPa.
(d), (e), (f) are scale up photographs of (a), (b), (c), respectively.

Conclusions

Not only muscle fibers but also intramuscular connective tissue was tenderized by high-pressure treatment in this study. In addition, pressure-dependent decrease of thermal stability and structural weakening of intramuscular collagen were observed. These results suggest that high-pressure treatment could cause some changes in intramuscular connective tissue, resulting in meat tenderization.

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