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The effects of pressure treatments with kiwi fruit protease on adult cattle semitendinosus muscle

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Abstract

The tenderizing effects of kiwi fruit protease on semitendinosus muscle were investigated. Adult cattle semitendinosus muscles were immersed in kiwi fruit protease (crude actinidin) at 20 °C for 15 min, and pressurized at 0–500 MPa and then heated at 80 °C for 15 min. The decreasing effects of the kiwi fruit protease-treatment with 0–500 MPa pressure on shear force and work done values were larger than those of the pressure-treated samples without kiwi fruit protease. Ultrastructures of the myofibrillar protein of the muscles treated with kiwi fruit protease showed the disorganization of the actin and myosin fillaments. The unheated and kiwi fruit protease-treated connective tissue, with pressure treatment (0–500 MPa), had little soluble α-chain collagen and after pressure treatment without kiwi fruit protease, soluble α-chain collagen was not obtained. © 2002 Published by Elsevier Science Ltd.

Keywords: Kiwi fruit protease; Semitendinosus muscle; Pressure and heat treatment; Tenderizing; α-Soluble collagen

1. Introduction

Tenderness is an important attribute of meat that affects its acceptability. Experiments in Australia (*Second symposium condition and meat quality in pigs*, 1971) showed that it was possible to tenderize both beef and mutton by subjecting the muscles to very high pressures for short periods. Pressures of 100 MNm^{-2} applied for 2–4 min reduced the shear force values [Warner-Bratzler (WB)] and caused severe contraction and disorganization of the muscles. Subsequently, it was found that combined pressure–heat (P–H) treatment (150 MPa at 60 °C for 30 min) effects a substantial decrease in shear force (Bouton, Ford, Harris, Macfarlane, & O'Shea, 1977). The ultrastructural studies revealed that P–H treatment disrupted both the thick and thin filaments, leaving voids in the M-line region (Macfarlane, McKenzie, & Turner, 1986). Rowe (1974) demonstrated that changes in meat toughness, resulting from contraction, can no longer be related only to changes in myofibrillar toughness, but must also include extensive changes in connective tissue toughness.

Proteolytic enzymes, such as ficin, papain, and bromelain, have been used in the tenderizing of meat. The action of the proteolytic enzymes is preferentially against connective tissue fibres. It should be emphasized that these enzymes do not attack native collagen, they act on the collagen as it is denatured by heat during cooking (Lawrie, 1998; section 10.3.4, pp. 244–246)

Actinidin is one of the plant thiol proteinases, such as ficin, papain, bromelain, and is present in the fruits of the chinese gooseberry or kiwi fruit (Actinidia chinensis; Kamphuis, Drenth, & Baker, 1985). Samejima, Choei, Ishioroshi, and Hayakawa (1991) showed that when beef pieces were immersed in crude actinidin solution at 4 °C, the cutting strength for raw and cooked meats decreased gradually with time of soaking, and scanning electron microscopy of meat treated with crude actinidin showed that it was more fragile than that of controls.

There are many papers describing tenderization of meat due to structural changes of the myofibrils caused by high pressure (Suzuki, Kim, Homma, Ikeuchi, & Saito, 1992; Suzuki, Watanabe, Iwamura, & Saito, 1990). But there are few papers describing the effects of pressurization on connective tissue. Beiken, Macfarlane,

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and Jones (1990) suggested, in a paper describing the effect of pressure during heat treatment on WB shear force values of beef muscles, that pressure treatment at temperature ranging from 40 to 80 °C had little or no effect on the background toughness. Suzuki, Watanabe, Ikeuchi, Saito, and Takahashi (1993) demonstrated no significant differences in the electrophoretic patterns of the isolated intramuscular collagen of control and pressurized muscles. Nishimura, Hattori, and Takahashi (1995, 1996) reported weakening of the intramuscular connective tissues, caused during extended ageing. Ueno, Ikeuchi, and Suzuki (1999) showed that the intramuscular connective tissues from bovine skeletal muscle exposed to high pressure (100–400 MPa) were comparable with those in muscle during ageing.

However, there are no papers describing the effects of pressure treatments with kiwi fruit protease on muscles and connective tissue (tendon). In the prsent study, we examine the effects of pressure treatments (0–500 MPa) with kiwi fruit protease by measuring WB shear force and work done values, and then those of kiwi fruit protease, on the myofibrils and connective tissue (tendon), by observing the microstructure, and by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

2. Materials and methods

2.1. Meat and tendon

Semitendinosus muscles were dissected from one cow at 2 years within 1 day post mortem and divided in the direction of fibrils into portions, about 7×5×5 mm. Achilles tendon, excised from adult cattle within 1 day of sacrifice, was dissected to remove adhering tissue and perichondrium and then shredded. The shredded tendons were washed in cold distilled water and 0.05 M Tris–HC1 (pH 6.8) at 4 °C, and small pieces, 5 mm in thickness, were tested.

2.2. Enzyme solution

The papain-like cysteine proteinases consist of papain, caricain, bromelain, actinidin, ficin, and aleurain, and the lysosomal cathepsins B,H,L,S,C and K. Most of these enzymes are relatively small proteins with Mr values in the range 20,000–35,000 (Turk, Turk, & Turk, 1997). Actinidin has been characterized by its activity against gelatin (optimum pH 4.0; McDowall, 1970). Kiwi fruit protease acted against telopeptide, but caused no significant cleavage of the triple helix domain of collagen (Sugiyama, Ohtsuki, Sato, & Kawabata, 1997). Actinidin has been shown to have many similarities to papain in kinetic behaviour and specificity. New Zealand kiwi fruit (Hayword) were purchased from the local market, homogenized, centrifuged at 13,000×g for 10 min, and then filtered (kiwi fruit protease, crude actinidin).

Purified enzyme, papain, ficin (Organo Teknika Co.), bromelain (Sigma Chemical Co.), actinidin (BioPur AG) were purchased. Ten millitres of the enzyme solutions were prepared by adding 10 mg of each respective enzyme to 0.2 mM formate buffer/20 mM EDTA/I10 mM cysteine/0.35% acetic acid, pH 3.3 and incubated at 20 °C for 40 mm to allow penetration of each enzyme into the muscles.

2.3. Pressure treatment

The dissected semitendinosus muscles were treated with or without kiwi fruit protease at 20 °C for 15 min. Then the processed samples were pressurized at 0–500 MPa in plastic films (50 mm in width and 80 mm in length) in a vessel 65 mm in diameter and 125 mm in depth, using the IHI ITP-80 type pressure treatment equipment at 0 °C (pressure-treated sample).

2.4. Mechanical measurement

The pressure-treated samples were heated at 80 °C for 15 min. WB shear force and work done values, of the processed samples, were measured with the Instron Universal Testing Machine as described by others (Bouton, Harris, & Shorthose, 1975).

2.5. SDS-PAGE

The effects of kiwi fruit protease on the connective tissue were analyzed by using SDS-PAGE. The shredded tendons were immersed in kiwi fruit protease at 20 °C for 15 min, washed in 20% NaCl and then treated with pressure at 0–500 MPa: But the pressure-treated samples were not followed by heating. Changes in the processed samples were compared with those of the untreated samples after pressure at 0–500 MPa (control), using SDS-PAGE. SDS-PAGE was carried out according to the procedure described by Laemmli (1970). The samples were dissolved in 1 ml of buffer A and B (1:1), A: 0.2 mM sodium formate/10 mM cysteine/20 mM EDTA (pH 3.3); B: 0.35% acetic acid/25% glycerol. Then 0.1 M Tris–HCl/1% SDS, pH 6.8 (50 μl), 2-mercaptoethanol (2 μl), 0.1% bromophenol blue (3 μl) were added to the solubiized sample solutions (50 μl) before heating at 90 °C for 10 min. Gels with 10% of polyacrylamide concentration were employed and stained with CBB (Coomassie brilliant blue).

2.6. Ultrastructural appearance

The effects of plant thiol proteinases on the myofibrillar protein were analyzed by observing the ultrastructural appearance. Samples were prepared as follows: the

semitendinosus muscles were incubated at 20 °C for 40 min with plant thiol proteinases, such as papain, ficin, bromelain, and actinidin, and were not followed by heating at 80 °C for 15 mm. The processed samples (1×1 mm) were fixed in 2% glutaraldehyde/0.1 M phosphate buffer solution, and then postfixed in 1% osmium tetraoxide (OsO4), in 0.1 M phosphate buffer (pH 7.4). After postfixation, the preparation was treated with a serial dilution of ethanol solution, decreasing from 50 to 100%, replaced with propylene oxide, and then embedded in TAAB 812. The fixed specimens were sectioned on a LKB-4800 Ultramicrotome, stained with uranyl acetate and lead citrate, and then examined by a scanning electron microscope (JEM-100CX).

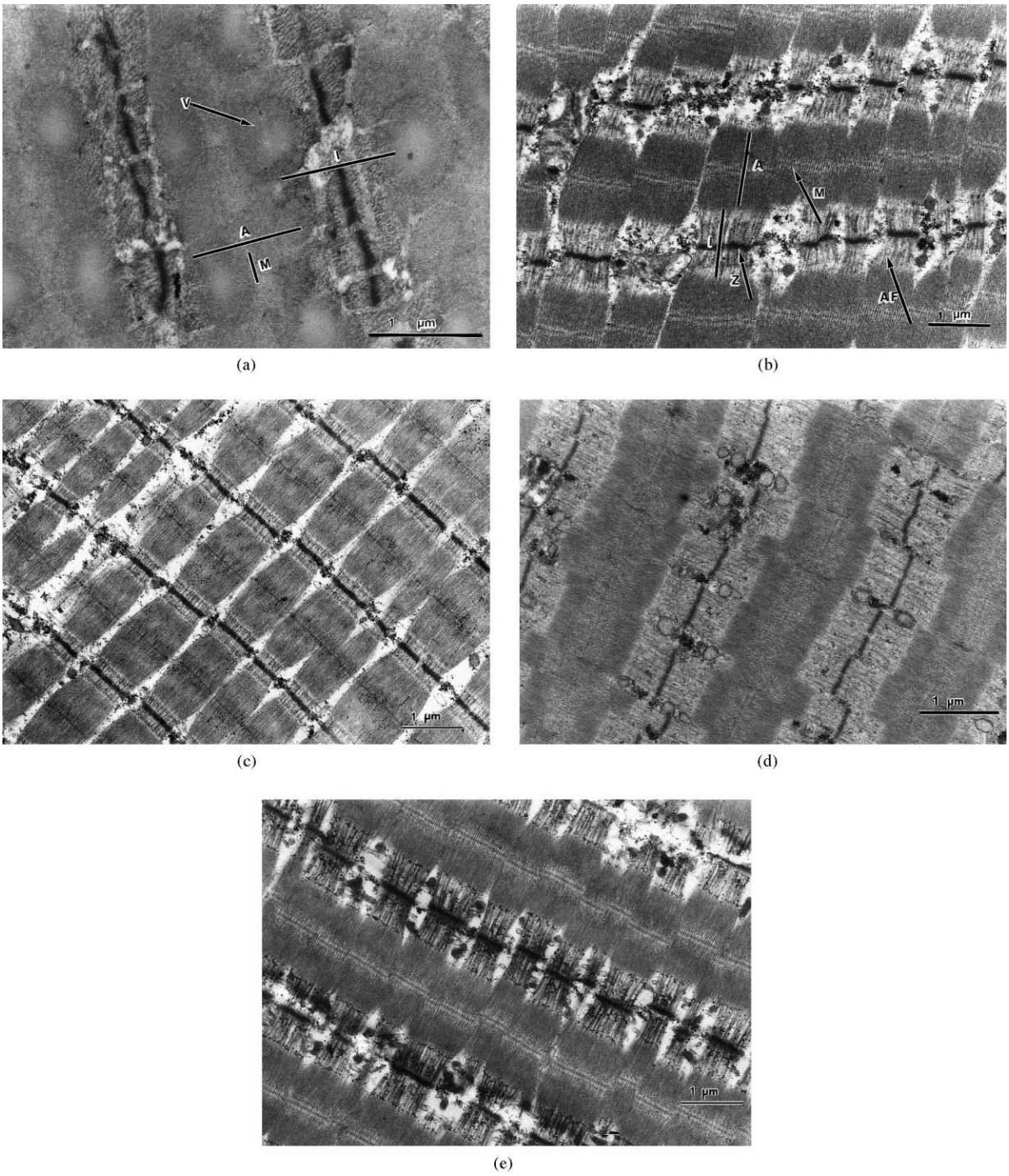

Fig. 1. Effect of plant thiol proteinases on the ultrastructure appearance of adult cattle semitendinous muscle (magnitude × 5000–10,000). A, papain treatment; B, actinidin treatment; C, ficin treatment; D, bromelain treatment; E, Untreated control sample.

Table 1
Effects of kiwi fruit protease on shear force and work done values of the adult cattle semitendinosus muscle

Pressure (Mpa)	Work done		Shear force	
	Treated (mJ)	Untreated (mJ)	Treated (N)	Untreated (N)
0	9.620±0.509	10.719±0.410	6.116±0.125	8.760±0.353
100	9.123±0.303	10.371±0.478	6.024±0.182	8.231±0.243
200	9.71 8±0.358	11.171±0.588	6.426±0.266	7.745±0.260
300	9.701±0.653	8.5440±0.647	6.209±0.332	6.307±0.305
400	9.510±0.692	11.663±0.695	5.947±0.513	7.342±0.751
500	7.224±0.755	12.411±1.207	5.040±0.395	7.756±0.960

mJ, mJoules, N, Newtons. Values: mean±standard deviation of five measurements.

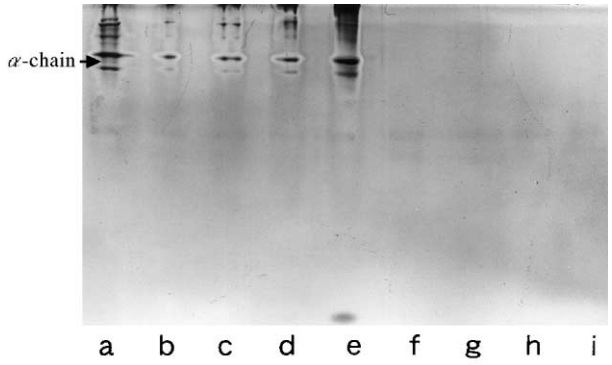

Fig. 2. (SDS-PAGE) assay of adult cattle achilles tendon with kiwi fruit protease: (lane a), control sample of type 1 acid soluble collagen from rat tail achifies tendon (Sigma Chemical Co.); (lane b), kiwi fruit protease treatment without pressure treatment; (lane c), kiwi fruit protease pressure treated at 100 MPa; (lane d), kiwi fruit protease pressure treated at 300 MPa; (lane e), kiwi fruit protease pressure treated at 500 MPa; (lane f), untreated control sample; (lane g), 100 MPa pressure treatment with no kiwi fruit protease; (lane h), 300 MPa pressure treatment with no kiwi fruit protease; (lane i), 500 MPa pressure treated with no kiwi fruit protease.

2.7. Statisical methods

The analysis of variance (two-way layout design) was used to determine statistical significance of difference at $P \leqslant 0.05$.

3. Results and discussion

3.1. Tenderizing effects of kiwi fruit protease on muscle

The effects of kiwi fruit protease on adult cattle semitendinosus muscle were analyzed by measuring WB shear force and work done values (Table 1). The means included five determinations. There were decreases in WB shear force and work done values of the adult cattle semitendinosus muscle. WB shear force values of pressure treatments at 0–500 MPa with kiwi fruit protease were significantly lower ($P < 0.01$) than those of pressure treatment at 0–500 MPa with no kiwi fruit protease. But there were no differences between 0 and 500 MPa in WB shear force and work done values of pressure treatments with or without kiwi fruit protease.

3.2. Electron micrographs of plant thiol proteinase-treated muscle

The effects of plant thiol proteinases, such as actinidin, papain, ficin, and bromelain, on the myofibrillar protein of adult cattle semitendinosus muscle were analyzed by observing the ultrastructure presented in (Fig. 1a–e). The papain effects (Fig. 1a) show that the M-line (M) and filaments of myosin of A-band (A) are mostly absent, and formed into relatively large voids (V). The actin filaments of I-band (I) of papain were disrupted and the disorganization effects of papain was larger than those of control samples (Fig. 1e). Actinidin (Fig. 1b) had fewer disaggregation changes in the actin control (AF). The myosin ifiaments of A-band (A) were almost intact. There are no electron microscopic differences between ficin-treated (Fig. 1c) and control samples. Actin filaments of I-band (I) of bromelain (Fig. 1d) were disaggregated, as compared with the control sample.

These findings indicated that the plant thiol proteinases affected the structure of the actin and myosin filaments of myofibrillar protein and that the plant thiol proteinases caused the degradation of actin and myosin ifiaments under unheated conditions.

3.3. Effects of kiwi fruit protease on native collagen

The pressure-treated connective tissue (temdon; 0–500 MPa), with or without kiwi fruit protease, were analyzed by SDS-PAGE (Fig. 2). The results show that the fragments of soluble α-chain collagen were not detected in the tendon after sacriflce (control sample, lane f), that kiwi fruit protease treatment without pressure had little soluble α-chain collagen (lane b) and the effect slightly increased with increase in the pressure range from 100 to 500 MPa (lanes c, d, and e), and that pressure treatments (0–500 MPa), with-out kiwi fruit protease, gave no soluble α-chain collagen (lanes g, h, and I).

These findings show that the decreasing effects of the connective tissue on WB shear force values are related not only to heated collagen during cooking, but must also include unheated collagen.

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