Effects of High Hydrostatic Pressure on Intramuscular Connective Tissue

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Background and Objectives

Meat tenderness has been resolved at least into two different components "actomyosin toughness" and "background toughness" The actomyosin toughness is the toughness attributed to the myofibrillar protein, whilst the background toughness is the toughness due to the presence of the connective tissue. Generally it is accepted that changes in the connective tissue during aging of meat are only slightly in comparison with those in the myofibrillar protein.

There are few papers describing the effects of pressurization on connective tissue as compared with those on myofibrillar proteins. Ratcliff *et al.* (1) showed that although pressure-heat treatment effectively eliminated the myofibrillar toughness (actomyosin toughness), the tenderness of the treated sample was limited by the connective tissue toughness (background toughness). Macfarlane *et al.* (2) also revealed that a transition attributed to F-actin was absent, but that attributed to the connective tissue was not changed in the thermograms of the pressurized muscle. Beilken *et al.* (3) suggested in a paper describing the effect of pressure during heat treatment on Warner-Bratzler shear force value of beef muscle that pressure treatment at temperature ranging from 40 to 80 ℃ has little or no effect on the background toughness other than to raise the temperature at which heat treatment alone produce a decrease in this toughness. In our previous report (4), we reported that no significant differences in the ultrastructure, electrophoretic pattern, thermal solubility and thermogram of DSC analysis of the isolated intramuscular collagen were observed among the control (untreated) and pressurized muscles.

Recently Nishimura *et al.* (5) suggested that the weakening of the intramuscular connective tissue, endomysium and perimysium, caused during extended aging correlated with meat tenderization using scanning electron microscopy. We investigated whether the similar changes as observed in the aged muscle were induced by pressure treatment or not. The extarctability and SDS-PAGE profile of proteoglycans (PGs) from the pressurized muscles were also examined.

Materials and Methods

Lean meat was excised from the shoulder part of a beef carcass 3 days after slaughter and stored in a freezer at -25 ℃. As required, it was tempered overnight in a cold room (2 ℃) and then cut into small pieces (50 mm × 50 mm × 30mm).

Pressurization of muscle

Each piece of muscle was vacuum-sealed in a polyethylene bag and transferred into a large polyethylene bag. The space between the bags was filled with crushed ice and water. Each bag was transferred to a pressure vessel, which was maintained at about 2 ℃, and pressure was applied at 100, 200, 300 and 400 MPa for 5min, using NBIP (Nikkiso ISOSTATIC Processor).

Scanning electron microscope (SEM) studies

Specimens for SEM were prepared by the method of Ohtani *et al.*(6), and examined with a SEM ABT-55 (Akashi Beam Technology, Japan).

Extractability and SDS-PAGE profile of PGs

PGs was extracted from the pressurized muscle by the method of Parthasarathy & Tanzer (7), and the amount of hexuronic acid in PGs was determined by the procedure of Bitter and Muir(8). SDS-PAGE of PGs was carried out on the 0.9-10% polyacrylamide gradient slab gels prepared by the method of Johnstone & Thorp (9) with a slight modification. After the run, gels were stained by the procedure of Vilim & Krajickova (10).

Results and Discussion

Scanning electron micrographs of the intramuscular connective tissues in the pressurized and aged muscles are shown in Fig. 1. During aging the structural weakening of the endomysium and perimysium proceeded, and the disruption of honeycomb structure was observed (Fig. 1-b). In the pressurized muscle, deformation of honeycomb structure of endomysium was accelerated with increase of the pressure applied to the muscle, and expansion of the hole of endomysium was observed in the muscle pressurized at 400 MPa (Fig.1-a). The amounts of PGs extracted from the pressurized and aged muscles are shown in Fig. 2. The changes in the extractability of PGs were not observed in the pressurized muscle, whereas the extractability of PGs decreased with the progress of the aging. The SDS-PAGE profiles of the PGs are shown in Fig. 3. The band with molecular mass over 3000 kDa seems to be main component in PGs. Changes in the density of this band were not observed in the PGs from the pressurized muscle, whereas the density of this band gradually decreased during the aging.

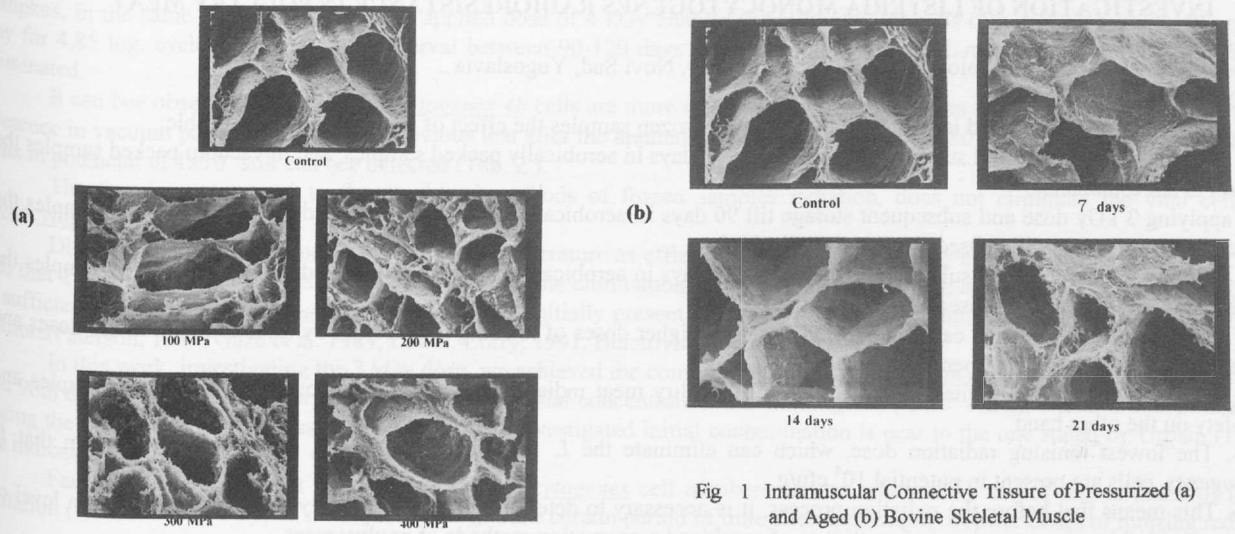

Fig. 1 Intramuscular Connective Tissure of Pressurized (a) and Aged (b) Bovine Skeletal Muscle

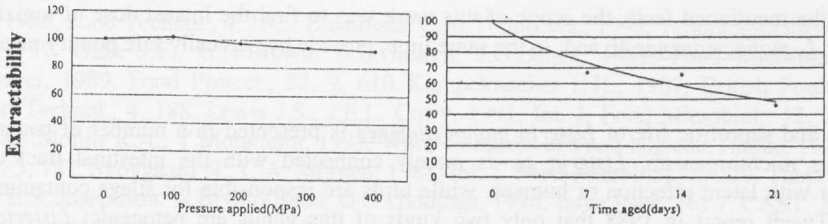

Fig. 2 Extractability of PGs from Pressurized and Aged Muscle (Extractability is expressed as % of that from control).

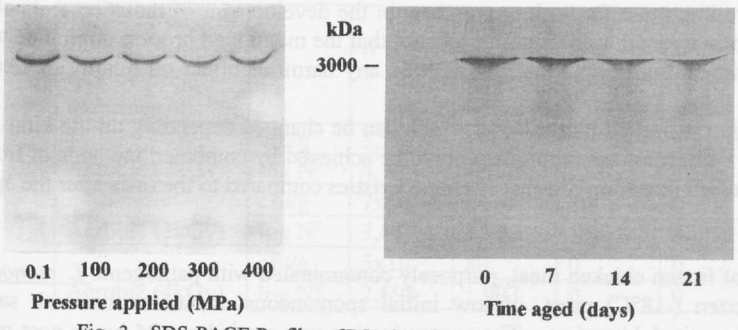

Fig. 3 SDS-PAGE Profiles of PGs from Pressurized and Aged Muscle

From the results, it seems that high pressure may have different effects on the intramuscular connective tissue than aging. The weakening of the membrane structure of connective tissue during storage may be induced by the degradation of PGs which stabilize the structure of the endomysium and perimysium due to the association with the collagen fibrils as suggested by Nishimura *et al.* (5). In the case of pressurized muscle, there were no relationship between the changes in the membrane structure of the connective tissue and the extractability of PGs. The difference in the extractability of the PGs between the pressurized and aged muscles may depend on the level of enzyme capable of degrading PGs both in the pressurized and aged muscles. Further studies will be required to clarify this problem.

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