# RELATIONSHIPS BETWEEN CONNECTIVE TISSUE AND MECHANICAL PROPERTIES OF NATURAL SHEEP CASINGS: EFFECTS OF CHRONOLOGICAL AGE

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## Background

In the meat industry, natural casings are well used in the manufacture of sausage. Natural casing varies, however, in quality especially in its strength and its elasticity. Hog and sheep casings from China are tougher than those from other countries (SAKATA *et al.*, 1998, NISHIUMI *et al.*, 1999). Natural casings are made from the submucosa layer of the small intestine of hog and sheep, which is obtained by the removal of the inner mucosus and outer muscular layers. The submucosa layer is composed of connective tissue largely organized by collagen fibers. In the preliminary study, we have demonstrated that the thermal and structural stabilities of collagen may determine the mechanical properties of the natural casings (NISHIUMI *et al.*, 2001).

## Objectives

It is assumed that the chronological age influences the connective tissue and mechanical properties of the casings. The aim of this study was to investigate the effects of age at slaughter on the relationships between connective tissue and mechanical properties of natural sheep casings. Structure and arrangement of collagen fibers, heat-solubility of collagen, heat-stable cross-link pyridinoline, and mechanical strength of lamb and sheep casings from New Zealand were evaluated.

## Methods

**Materials:** Desalted natural lamb and sheep casings (20-22 mm in diameter) from New Zealand were used for the following analyses.

**Mechanical strength measurement:** Mechanical strength of the casings was measured by a Rheometer (Fudoh NMR-2002J, Tokyo), which was assessed as a peak breaking strength using a cylindrical planger 3-mm in diameter that has been inserted into the casings.

**Scanning electron microscopy (SEM):** Structure and arrangement of collagen fibers were observed using a scanning electron microscope (Hitachi S-430, Tokyo). The SEM specimens were prepared according to the cell-maceration method (OHTANI *et al.*, 1988). Small pieces of the casings fixed in 2% paraformaldehyde-2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for more than 3 days were immersed in 10% NaOH for 5 days and then rinsed in distilled water for 3 days. This was followed by conductive-staining with tannin-osmium, dehydration in an ethanol series, and drying by *t*-butyl alcohol lyophilization. The dried specimens were mounted on metal stubs, coated with gold and observed for the architecture of collagen fibers in casing under SEM with an accelerating voltage of 15 kV.

**Heat-solubility of collagen assay:** Freeze-fractured casing in liquid nitrogen was heated for 70 min at 77C in PBS and separated into heat-soluble and insoluble fraction as outlined by HILL (1966). Individual fractions were acid hydrolyzed in 6 N HCl for 24 h at 110°C and hydroxyproline was quantitated. Collagen content was calculated by multiplying the hydroxyproline content by 7.25 (insoluble collagen) and 7.52 (soluble collagen). Percentage of soluble collagen in each casing sample was expressed as the heat-solubility of collagen.

**Pyridinoline assay:** Pyridinoline was measured according the method described by ARAKAWA *et al.* (1992). After acid hydrolysis and evaporation *in vacuo*, the dried hydrolysates were dissolved in 3 ml of distilled water and filtered through a chromatodisc (pore size 0.45 μm). A part was removed for hydroxyproline determination, and the remainder was subjected to pyridinoline assay. An isocratic liquid chromatography procedure was used to fluorescently quantify the pyridinoline in the casings. Pyridinoline standard was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo). Pyridinoline concentration in collagen was calculated assuming collagen had a molar mass of 300,000 g/mol.

## Results and Discussions

As shown in Table 1, lamb casing was significantly tender than sheep casing ($P<0.01$), which indicates that toughness of casing increased with an increasing animal age. Total collagen content of lamb casing was similar to that of sheep casing, whereas the lamb casing contained much more heat-soluble collagen than sheep casing did, which resulted in a significant high heat-solubility of collagen in the lamb casing (Table 1). Sheep casings possessed considerably more heat-stable collagen than lamb casings. The heat-solubility of collagen, in other words, thermal stability of collagen, is related to intermolecular cross-linking of collagen, collagen type, and size and arrangement of collagen fibers (BAILEY & LIGHT, 1989). We also investigated a heat-stable mature cross-link of collagen, pyridinoline in these casings. The pyridinoline cross-link was significantly more present in sheep compared to lamb (Table 1). In casing collagen, pyridinoline cross-linking with chronological age may induce the structural and thermal stability, which may result in increasing toughness of the casing.

SEM demonstrated the size and arrangement of collagen fibers and also thickness of the casings (Fig. 1). The arrangement of collagen fibers was almost similar in both casings, but collagen fibers in sheep were somewhat thicker than those in lamb (Fig. 1-A & B). In addition, sheep casings were considerably thicker than lamb (Fig. 1-C & D). These structural differences may also influence toughness of the casings.

## Conclusions

Mechanical property of natural casings increased with chronological age. Age-related changes in characteristics of the casing collagen were also found. Both structural and thermal stabilities of collagen may contribute in determining the textural quality of casing.

## References

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Table 1. Comparison of breaking strength, heat-solubility of collagen and pyridinoline content between lamb and sheep casings

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|---|
| Casing |
| Breaking Strength |
| Heat-solubility |
| Pyridinoline content |
| sample |
| (g) |
| of collagen (%) |
| (mol/mol collagen) |
| Lamb |
| 299± 85a* |
| 12.3±0.42a |
| 0.046±0.0031a |
| Sheep |
| 407±120b |
| 3.32±0.22b |
| 0.128±0.0087b |

* Means with different letters indicate significantly different (P<0.01).

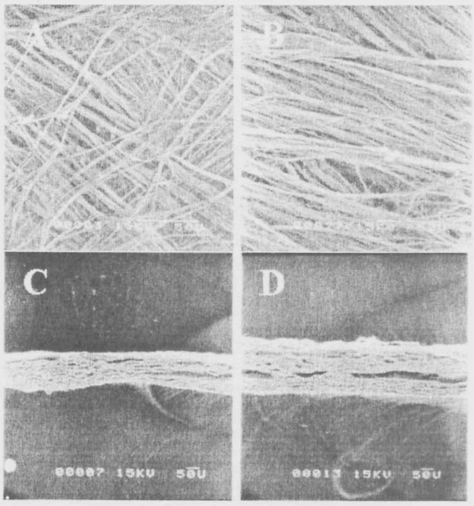


**Fig. 1.** SEM of collagen fiber arrangement in lamb and sheep casings.
A and C, lamb; B and D, sheep