3-P27

HISTOLOGICAL AND BIOCHEMICAL EVALUATION OF CONNECTIVE TISSUE OF NATURAL HOG AND SHEEP CASINGS

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Background:

In the meat industry, natural casings are well used in the manufacture of sausage. The strength and elasticity of casings must be adequate. Casings too tough are not acceptable for marketing purposes. Hog casings from China are tougher than those from the USA (Sakata et al., 1998). 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 largely composed of connective tissue, collagen fibers and minor elastin fibers in the blood vessels. The mechanical properties of casings are thus likely affected by the degree of elastin accumulation and mechanical stability of collagen which depends not only on intermolecular cross-linking of collagen but as well, the size and arrangement of collagen fibers (Rowe, 1981, Nishimura et al., 1996). To date, little attention has been directed to the evaluation of connective tissue components in natural casings.

Objectives:

Study was made to elucidate the characteristics of connective tissue components of natural hog and sheep casings. Examination was made of the morphology and structural arrangement of collagen and elastin fibers by light and scanning electron microscopy. Biochemical evaluation was made of collagen and elastin. Preliminary data are presented.

Methods:

Materials: Salted natural hog casings (32-34 mm in diameter) from China and Japan and sheep casings (20-22 mm) from China and Australia were used. They were washed and desalted in running water for 3 h for histological and biochemical analysis.

Histological analysis: Duplicate samples from casings (each 5 cm long) were used. Each sample was fixed in 10% formalin-PBS for more than 24 h. The large samples were cut into small pieces with transverse area of approximately 1 cm^2, stained for elastin with modified Verhoff's Van Gieson (Elastin Stain Kit, Sigma, St. Louis) and obeserved under a light microscope (BH-2, Olympus, Tokyo). Small pieces fixed in 2% paraformaldehyde-2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for more than 3 days were processed for scanning electron microscopy by the cell-maceration method (Ohtani et al., 1988) immersed in 10% NaOH for 5 days and rinsed in distilled water for 3 days. This was followed by conductive-staining with tannin-osmium (Murakami, 1974), dehydration in an ethanol series, and drying by *t*-butyl alcohol lyophilization (Inoue & Osatake, 1988). The dried specimens were mounted on metal stubs, coated with gold-palladium and observed for internal and external architecture of collagen fibers in casings under a scanning electron microscope (S-2380NS, Hitachi, Tokyo) at accelerating voltage of 15 kV.

Biochemical analysis: Aliquots of casings freeze-fractured in liquid nitrogen were used for elastin content determination. Other samples were dried and defatted in a 2:1 chloroform-methanol solution. Dried-defatted matter (DDM) was weighed for moisture and fat loss determination and then powdered. For collagen estimation, powdered DDM was heated for 70 min at 77℃ in PBS and separated into heat-soluble and insoluble fractions as outlined by Hill (1966). Individual fractions were hydrolyzed in 6N HCl for 24 h at 110 ℃ and hydroxyproline was quantitated (Bergman & Loxley, 1961). Hydroxyproline was converted to insoluble collagen using a factor of 7.25 and to soluble collagen, with a factor of 7.52 based on the absence of elastin (Cross et al., 1973b). Percentage of soluble collagen in each casing sample was expressed as the heat-solubility of collagen. Elastin was isolated from freeze-fractured casings by extraction with 1.1 M KI in 0.1 M phosphate buffer (pH 7.4) and selective hydrolysis with 0.1N NaOH for 50 min at 98℃ was carried out to remove the collagen portion of connective tissue according to Cross et al. (1973a). The remaining residue, elastin, was hydrolyzed and hydroxyproline content was determined as above. Elastin content was found by multiplying hydroxyproline content by 66.225 based on the amino acid composition of intramuscular elastin (Cross et al., 1973a). Elastin and collagen content was expressed as mg/g on a DDM basis, owing to the considerable variation in moisture % of casings subsequent to washing in running water.

Results and discussion:

By light microscopy, each casing was found composed primarily of numerous layers of sheets of crimped collagen fibers with crisscross arrangement. Differences in gross organization of the collagen fibers were not observed, but the outer sheet of sheep casings from China contained more stretched collagen fibers and was packed more densely compared to that from Australia. Elastin accumulated in blood vessels such as arterioles and venules. Fine elastin fibers, apparently capillaries, were scattered sparsely among the predominately present collagen fibers. The so-called "whiskers" observed microscopically as large projections of blood vessels and like navel strings in appearance could be seen in all casings. The morphology, localization and density of elastin fibers for casing samples, thus appear essentially the same.

Scanning electron microscopy demonstrated a three-dimensional arrangement of collagen fibrils. On internal surfaces of casings, numerous holes, possibly lymph follicles, were observed in all cases. The arrangement of collagen fibers on internal surfaces of sheep casings differed from that of hog casings, fibers of the former having the same direction while those of the latter were areolar. The external surface was composed of thin sheets, like textiles, organized of flat collagen fibers or fiber bundles and was the same for all casings. Remarkably, sheep casings from Australia possessed relative fine collagen fibers of loosely packed fibrils while the collagen fibers of sheep casings from China were similar to those of hog casings.

Hog and sheep casings contained approximately 87-98% collagen and 2% elastin as determined from Table 1. Elastin content of casings has yet to be determined. Considering that intramuscular elastin (around 2% elastin in intramuscular connective tissue, Nishiumi et al., 1995) is similar to that of the casing elastin, elastin may be associated with blood vessels and the low content of elastin in the present study would not likely affect the mechanical properties of natural casings but possibly account for "veiny" casings of well-developed blood vessels. The sheep casings contained as much elastin as hog casings, which is consistent with a recent morphometrical study (Bakker et al., 1999). But elastin content of hog casings from China and Japan was found to differ significantly. As shown in Table 1, heat-solubility of collagen for natural casings was relatively low (3.87% for Japanese hogs and 7.14% for Australian sheep) compared to intramuscular connective tissues (Nishiumi et al., 1995). This possibly may be indication of the removal of labile collagen during casing manufacture. Australian sheep casings contained significantly more labile collagen compared to others. The heat-solubility of collagen, that is, the heat stability of collagen, is related to intermolecular cross-linking of collagen, collagen type and size and arrangement of collagen fibers (Bailey & Light, 1989). Sheep casings from China may thus possess considerably more heat-stable collagen than Australian sheep.

The present results at least provide indication that the heat-solubility of collagen is related to the size and arrangement of collagen fibers, and thus possibly may have some effect on mechanical properties of casings. Relationships among thermal behavior of collagen fibers, types and cross-linking of collagen and mechanical properties of natural casings should be clarified in greater detail.

Conclusions:

Natural hog and sheep casings are largely composed of collagen organized in many layers of sheets of collagen fibers and minor elastin in blood vessels. The morphology, localization and density of elastin fibers and amounts of elastin for various casings are essentially the same. Elastin thus would not likely contribute to the strength of natural casings. The size and arrangement of collagen fibers and heat-solubility of collagen differ according to the casing and thus may determine the mechanical properties of casings.

Pertinent literature:

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Table 1. Mean elastin, soluble and insoluble collagen content and heat-solubility of collagen in natural hog and sheep casings

Casing sample	Elastin content(mg/g)	Soluble collagen content (mg/g)	Insoluble collagen content (mg/g)	Heat-solubility of collagen(%)
Hog, China	22.03 ± 1.59^{b}	38.77 ± 4.24^{ab}	946.63 ± 46.64^{b}	3.93 ± 0.38^{a}
Hog, Japan	16.24 ± 0.71^{a}	35.71 ± 2.02^{a}	883.52 ± 34.47^{b}	3.87 ± 0.27^{a}
Sheep, China	23.88 ± 1.23^{b}	44.00 ± 2.33^{b}	919.87 ± 44.03^{b}	4.55 ± 0.48^{a}
Sheep, Australia	22.79 ± 2.14^{b}	62.68 ± 5.46^{c}	811.01 ± 35.06^{a}	7.14 ± 0.73^{b}

[a-c] Means with different letters indicate significantly different ($P<0.01$).