MODELING CHANGES WITH AGEING IN THE MECHANICAL PROPERTIES OF BEEF CONNECTIVE TISSUE

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Background

Perimysium connective tissue is known to play an important role in the variations in meat toughness. It is a network of collagen fibers organized in ply and embedded in a ground substance made of various proteoglycans (Eggen, Malmstrom, Sorensen and Host 1997) which constitute the matrix of the tissue. During ageing, there is a weakening of the perimysium which separate into collagen fibers and fibrils. Whereas collagen remains unchanged at the molecular level, however, during ageing, there is a degradation of proteoglycans. This degradation as been put forward to explain the weakening of the perimysium connective tissue during storage. Nevertheless there are no data on the mechanical properties of this matrix and of these changes with treatments.

Objectives

The aim of this study was to quantify the mechanical changes occurring in the matrix of proteoglycans of perimysium connective tissue during storage.

Materials and methods

Muscles: *Semimembranosus* muscles were taken from a Charolais - Frisonne cow (3 ½ years old). The muscles were removed from the carcass at 24h *post-mortem*. They were divided into several parts which were vacuum packed and stored at 4°C for 2 or 14 days and then frozen.

Measurements :
All the samples were thawed in water at 10°C. Sheets of perimysium (approximately 10 mm long x 4 mm wide) were dissected and tested with a micro-tensile device developed in this laboratory, for the determination of breaking stress, breaking strain, Young's modulus, breaking energy and total energy. The width of the samples were determined using a microscope and the thickness determined with a Mitutuyo micrometer under a force of 0.2N applied on the whole surface of the sheets. During the tensile test the samples were immersed in meat drip. The procedure for handling samples is similar to that described by Lewis and Purslow (1989). After dissection, strips of perimysium were glued on aluminum foil frames with cyanoacrylate glue. The samples on the aluminum frames were then fixed on the micro-tensile device so that the direction of tensile strain corresponded to the direction of muscle fibers.
The extension rate was 130 µm/s. The software applied a slack toe correction to the force – displacement curves to remove the part of the displacement where collagen fibres are just unfolded. The Young's modulus was calculated as the slope in the straight region of the stress – strain curves.

Statistical analysis :
For all mechanical variables means were obtained from 80 to 120 measurements. Data were analysed using the general linear model procedure of SAS Software (SAS/Stat Cary, NC: SAS Institute Inc., 2000).

Mechanical model

The purpose of this model was to quantify the changes in Young's modulus and Poisson ratio of the matrix during ageing. The model proposed in this paper combines laminated membrane theory and reciprocal identification method to estimate the Young's modulus and Poisson ratio of the matrix. Evolutive anisotropy of the tissue due to the reorientation of collagen fibres with the perimysal strip elongation is taken into account from measurements of the variation with strain of the angle between collagen fibres and the direction of strain. As a first approximation, the model does not account for the crimping effect along the collagen fibres.

Model description:

Four engineering constants are needed to describe the mechanical behaviour of a ply; the longitudinal Young's modulus E_L, the transversal Young's modulus E_T, the longitudinal Poisson ratio ν_{LT} and the longitudinal shear modulus G_{LT}. They are expressed in terms of engineering constants of the constituents and fiber volume fraction using a parallel system for E_L, ν_{LT} and a series system for E_T and G_{LT}. Then the on-axis stiffness matrix [Q] of each ply can be computed and the off-axis stiffness matrix [Q(θ)], expressed in terms of [Q] and a transformation matrix [T(θ)]. Next, laminated membrane theory is used to derive the mechanical behavior of the perimysal strip made of two plies lying at +θ and - θ to the muscle fiber direction (Christensen, 1991). As expected in the case of tensile test, the shear stress in a ply is balanced by the oppositely directed shear stress in the second ply, such that the net shear force over the tissue is cancelled out. The equations of in-plane behavior of the tissue are not linear as usual because of the dependency of fibers orientation on the strip elongation; so iterative methods must be used to compute the stress-strain curves. As the model does not account for the fibers crimping, the initial non-linear region of the tensile curve can not be described.

Reciprocal identification method:

It has been assumed according to Liu, Nishimura and Takahashi (1994) that the collagen of intramuscular connective tissue remains unchanged at the molecular level during *post-mortem* ageing of meat. The model is also based on the assumption that the matrix is mechanically isotropic, which is a simplification as proteoglycan matrix is not amorphous. The following data were used for the simulations: collagen fiber Young's modulus E_f= 1000 MPa, collagen fiber Poisson ratio ν_f = 0.5, fiber volume fraction V_f=8%. For given values of matrix Young's modulus E_m and Poisson ratio ν_m, a numerical stress-strain curve of the perimysal strip was computed and the gap between numerical and experimental curves was evaluated. Discrepancies were reduced by adjusting E_m and ν_m. The fitting were done on part of the stress-strain curves limited by strain = 0 and breaking strain.

Results and discussion

The data in Table 1 show the overall changes in mechanical properties of perimysium sheets due to storage. Significant reductions were observed in breaking stress and Young's modulus.

Duration of storage (Days)	Breaking Stress (MPa)	Breaking strain	Modulus (MPa)	Beaking energy (mJ)	Total energy (mJ)
2	0.76 *a*	0.77 *a*	1.34 *a*	2.9 *a*	5.2 *a*
14	0.51 *b*	0.70 *a*	1.05 *b*	2.6 *a*	4.1 *a*

Table 1: Mechanical properties of perimysium sheets
In each column the values followed by different letters are significantly different at a 5% level.

The results of the modeling are given in Table 2. It appeared (data not shown) that when both Poisson ratio and modulus of the matrix were adjusted freely by the software to give the best fit of the stress – strain curves then the final value of the Poisson ratio was systematically negative which means that during the tensile test there is an expansion of the matrix volume. It is not a classical behavior of materials although already mentioned by Vincent (1990). So, the Poisson ratio of the matrix was fixed in the range $0 < \nu_m < 0.5$.

Storage (days)	N	Modulus (MPa)
2	80	0.47 *a*
14	119	0.38 *b*

Table 2 : Matrix modulus obtained from modeling.
In each column the values followed by different letters are significantly different at a 5% level

There was a reduction of about 20% of the matrix modulus between 2 and 14 days of storage. The Poisson ratio being zero at 2 and 14 days.

Conclusions

The results obtained in this study agreed with those from Nishimura, Hattori and Takahashi (1995, 1996) in beef, which showed that there is a progressive weakening of perimysium connective tissue during storage explained by a degradation of proteoglycans. This study shows that after 14 days of storage there is a a 30% reduction in breaking stress and a 20% reduction in Young modulus measured in tension. A similar decrease in breaking stress has been found by Lewis, Purslow and Rice (1991). A mechanical model based on the composite theory has been developed in order to quantify the mechanical changes occurring in the proteoglycans matrix during storage. It appears that a decrease of the modulus of the matrix by 20 % associated with a null Poisson ratio can explain the changes observed at the level of the perimysium network between 2 and 14 days of ageing.
An improvement of the model is needed as it does not take into account the unfolding of collagen fibers and assumed that the matrix was isotropic.

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