MUSCLE, AGE AND SEX EFFECTS ON TOTAL PYRIDINOLINE CROSS-LINKS CONTENT AND BEEF TENDERNESS

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Introduction

After ageing which reduce the resistance of muscle fibres, and in absence of cold shortening and pH defaults, the variations of meat tenderness are mainly determined by connective tissues features. Before heating, collagen fibres have a very high elastic modulus, but after heating above 58-65°C collagen is denatured, contracts and becomes rubber like. In this state the mechanical properties of collagen fibres are determined by their content in cross-links. In collagen there are different types of cross-link which are more or less thermo stable (Bailey, 1983; Reiser *et al.*, 1992; Eyre and Wu, 2005). The increase in number of cross-links per mole of collagen with animal age explains partly the increase in meat toughness observed in old animals (Bailey, 1989). Nevertheless, the correlations between the number of cross-links per mole of collagen and meat tenderness among different muscles or animals may vary greatly from relatively high (r=0.82: Bailey, 1989) to non significant absolute values (Shimokomaki *et al.*, 1972). McCormick (1999) concluded that mature cross-links and collagen concentration have an additive effect on the toughening of meat. To take into account the influence of both parameters Maiorano *et al* (2001) suggested considering the cross-link concentration per mass of meat. Lepetit (2007) demonstrated that the elastic modulus of the connective tissues of rest length aged meat is proportional to the sum of all cross-links per volume of meat, each cross-link being weighted by its functionality. The functionality of a cross-link is defined as the number of chains meeting at the cross-link. This work also showed that the number of pyridinoline cross-links per volume of meat has by itself a potential to explain the mean effects of age, sex and muscle on shear force or tenderness.

The purpose of this work is to determine the percentage of shear force variation explained by pyridinoline cross-links in an experiment involving different muscles from bulls and cows of different ages.

Materials and Methods

Gluteus Medius (GM), Longissimus dorsi (LD) and Semitendinosus (ST) muscles from 4 young Limousine bulls (14 to 19 months old) and 4 Limousine old cows (8-10 years old) were removed from carcasses 24 h after slaughter, vacuum packed, stored 14 days at 4 ± 2°C and then frozen. Samples were thawed in water bath at 10°C for 1 h just before measurements. For pyridinoline determination, 100g of muscle were frozen at -20°C then ground, freeze dried and ground again.

Shear force measurements:

Pieces of meat (3cm thick in the direction perpendicular to muscle fibres) were grilled simultaneously on both sides with a grill model Infragrill E (Sofraca France) until a core temperature of 55 ± 1°C was reached. After heating, due to the equilibration of temperatures within the samples the core temperature reached 62 ± 1°C. Cooking loss were measured. Shear force was determined on samples (1x1x3 cm) sheared perpendicularly to muscles fibres direction with a Warner Bratzler cell fitted on a universal testing machine. Shear force is a mean from 10-12 samples. The sheared samples were frozen for pyridinoline determination on cooked meat.

Immunological determination of pyridinoline:

Pyridinoline cross-links were assessed after acid hydrolysis of 500 mg lyophilised raw or cooked meat in 20 ml of 6 M HCl heated at 110 °C overnight in a screw-capped glass tube. The resulting hydrolysate was centrifuged at 16,000g for 5 min and 300µl of the supernatants were added with 300 µl of 6M NaOH and 300 µl of 1M Tris. Final pH was adjusted between 7 and 8 with a few microliters of 6M HCl or NaOH. Pyridinoline cross-links (Pyridinoline + Deoxypyridinoline) were determined by the enzyme linked immunoassay Metra Pyd EIA kit (Quidel corporation, USA) as described in the guide of the kit. Previous assays had evidenced no interfering effect of the 1/10 diluted muscle extract on the Pyridinoline cross-links determination (results not shown).

Results and Discussion

The means value of shear force of muscles increases in the order *LD*<*GM*<*ST* in each group of animal age, which is the same for total pyridinoline cross-links content (Figure 1). The tender (*LD*) muscle of old cows is tougher than the toughest (*ST*) muscle of young bulls. When considering the mean values by muscles and age there is a strong correlation between mean shear force and the mean total pyridinoline cross-links content expressed as nMole/mg of lyophilised sample, measured either on raw muscles or on cooked meat. The pyridinoline cross-links content of cooked meat is shifted to higher concentrations due to dry matter loss in cooking loss. When considering all individual muscles and animals the correlation between shear force and pyridinoline cross-links content is 0.79 (N=24, P < 1 %). In each muscle the correlations between shear force and total pyridinoline cross-links content decreases with the content in pyridinoline cross-links in this muscle. Correlations are: *ST*, 0.87, P < 1 %; *GM*: 0.73, P < 1 %; *LD*: 0.59 P < 5 %.

The mean cooking loss of all three muscles is higher (33±1%) in old cows than in young bulls (28±1%) and this is approximately in the same order of variation than the variation of the mean amount of total pyridinoline cross-links content: (0.038 ± 0.008 nMole/mg lyophilised tissue for old cows, 0.025 ± 0.004 for young bulls).

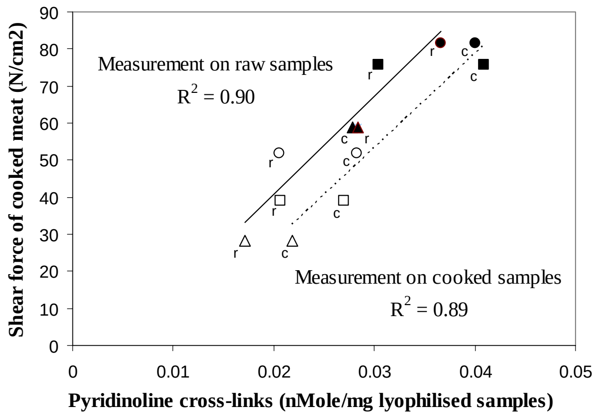

Figure 1: Relationship between total pyridinoline cross-links content and shear force of cooked meat.
Triangle: *Longissimus Dorsi.* Square: *Gluteus Medius.* Circle: *Semitendinosus.*
Open symbols D £ O : Young bulls. Dark symbols 5 ¢ l: Old cows.
The signification of the indices near the symbols is:
r : Pyridinoline measured on raw muscles. c: Pyridinoline measured on cooked meat

Conclusions

Variations of pyridinoline cross-links amount and tenderness of the 3 muscles are in agreement with data of McCormick (1999). The total pyridinoline cross-links content measured either on raw muscles or on cooked meat and expressed as Mole/masse of sample predict about 60 % of the variations of tenderness associated to muscle, sex and age effects. A better prediction is expected if all cross links are considered. The prediction by pyridinoline of tenderness variations of a muscle decreases according to the average amount of pyridinoline in this muscle. The pressure developed by connective tissues during cooking is proportional to the amount of collagen cross-links (Lepetit, 2007). This explains why cooking loss increases with age in the same order of magnitude than the increase in total pyridinoline content.

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