ENDOGENOUS MUSCLE CHARACTERISTICS RELATED TO MEAT TOUGHNESS

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SUMMARY: Correlations were done to determine which muscle characteristics from *Longissimus* muscle of 8 Charolais bulls (26 months old) might explain the myofibrillar toughness of fully aged meat. The oxidative status and the initial level of calpastatin, were positively correlated while, at 1 hour, levels of μ- and m-calpains, cysteine and serine proteinase inhibitors and pH were negatively correlated, as was the rate of pH decline, with ultimate toughness. However, the individual correlation coefficients between the characteristics and meat toughness were less than 0.65. Therefore, it is difficult to classify correctly meat toughness because of the interactions of those characteristics or interventions of other characteristics.

INTRODUCTION : After the death, myofibrillar structure of the muscle changes progressively during storage and these changes are responsible for meat tenderisation. Meat tenderness is probably the most important organoleptic quality attribute for consumers but often the most variable. To reduce this variability, a better understanding of endogenous muscle characteristics implicated in meat ageing is necessary. Our aim is to find the endogenous muscle characteristics which explain the differences in ultimate toughness from the same muscle from cattle of similar breed, sex and age.

MATERIALS AND METHODS [1] :

Animals and sampling : The *M. longissimus* (Ld) from 8 cross Charolais bulls, aged 24 to 30 months, were excised and kept at 12°C for the first 24 h *post mortem* and then at 4°C for up to 14 days.

pH determination : The pH was determined from 2 g of muscle homogenized in 5 mM iodoacetate.

Muscle metabolism : Lactate dehydrogenase (LDH) and Citratesynthase (CS) enzymes were from 1 g of muscle, homogenized in 0.063 mM glycylglycine, 0.5 mM saccharose, 6.2 mM EDTA, 125 mM NaF, 5 mM DTT, pH 7.6. After 1 hour's extraction at 20°C and centrifugation at 5000 x *g* for 15 min, the activities were measured, using 2.5 mM pyruvate and 10 mM oxaloacetate as substrates respectively.

Cysteine and serine proteinase inhibitors : The activities of cysteine and serine proteinase inhibitors were from 20 g of muscle and determined with papain and trypsin respectively.

Calpain and calpastatin levels : From an extract of 20 g of muscle, the two calpains were separated from calpastatin using an hydrophobic column and were themselves separated by ion exchange chromatography. Activities were determined using 125 μM suc-leu-tyr-4-methyl-7-coumarylamide.

Rheological measurements : The resistance of the myofibrillar structure was measured as the stress at 20% compression in raw meat determined from 1 to 14 days.

Data analysis : The rate of decline of pH was determined as $(pH_{24} - pH_1)/23$ and the ultimate resistance was determined from an exponential curve with time *post mortem*.

RESULTS AND DISCUSSION :

Relationships between measured characteristics and ultimate resistance ($p<0.1$)

Values are linear correlation coefficients for 8 muscles

Characteristics	Linear correlation coefficient
Calpastatin at 1 hour pm	0.65
Citrate synthase	0.65
Rate of decline of pH	-0.62
Serine proteinase inhibitors	-0.61
pH at 1 hour pm	-0.59
μ-calpain at 1 hour pm	-0.53
Cysteine proteinase inhibitors	-0.53
m-calpain at 1 hour pm	-0.46

Calpastatin at 1 hour pm was the best correlated with the ultimate toughness: the richer the muscle was in this inhibitor, the more resistant was the meat. A role for calpastatin in meat toughness seems evident in the literature but there is still no accepted view as to which time *post mortem* calpastatin should be measured. Calpastatin at 24 hours, but not at 1 hour, was found to be a valuable predictor for meat ageing [2]. However, in this study, calpastatin at 1 hour explained 42% of the variability of myofibrillar toughness, a similar level to that reported for calpastatin at 24 hours [2].
The action of calpastatin is unclear. For some authors, calpastatin acts in meat ageing as an inhibitor of calpains [3] but for others [4], because it is not an inhibitor at the pH of meat, it is viewed as a substrate of calpains thus having a high correlation with proteolysis and toughness.
CS activity was correlated with the ultimate resistance: more 'red' muscles were tougher after ageing. Generally [5], extreme muscle types I, IIa and IIb give tender meat and intermediates tougher meat but, for any given muscle, the importance of carbohydrate metabolism to tenderness is not clear. Moreover, most typing has been done by histology and is sometimes contradictory. In this study, oxidative level, determined biochemically, explained 42% of the variability and could be a predictor of toughness but difficult to explain why because, generally, red muscles have more proteolytic enzymes than white [5].
The rate of pH decline was correlated with ultimate resistance: with higher rates of pH decline, the less resistant the muscle; emphasizing the importance of *rigor mortis* development to tenderness [4].
Serine proteinase inhibitor activity was correlated with the ultimate resistance: the more there was of these inhibitors, the more tender the meat. No publication has reported a correlation between these endogenous inhibitors and meat tenderness and no serine proteinases have been isolated from bovine muscle. However, exogenous serine proteinase inhibitors affect meat toughness [6].
The pH at 1 hour pm was correlated with the ultimate resistance: the higher was the pH at 1 hour, the less tender was the meat. Most authors agree with this result but disagree that a single measure of pH could be a good predictor of meat toughness [7 & 8].
The initial level of μ-calpain was correlated with the ultimate resistance: the richer the muscle was in μ-calpain, the less resistant it became. The fundamental role of this enzyme in meat ageing is now accepted by many meat scientists. However, in this study, the variability in μ-calpain accounted for only 30% of tenderness and its value as a predictor of tenderness is limited.
Cysteine proteinase inhibitor activity was correlated with the ultimate resistance: the more there was of these inhibitors, the more tender the meat. The activity of cystatin alone accounted for 10% [2] and, in this study, with all cysteine proteinase inhibitors, 25% of the variability in toughness.
The level of m-calpain at 1 h pm was correlated with ultimate resistance: the richer the muscle was in m-calpain, the less resistant it became. The importance of this enzyme in tenderness, compared to μ-calpain, has not been established. Here, m- was as important as μ-calpain in predicting ageing [9].
The most important muscle characteristics in meat toughness were found to be the oxidative metabolism and the level of inhibitors and enzymes. However, simple correlation coefficients explained, at best, 42% of toughness variability. It is likely then that several muscle characteristics are involved and interact in a complex way to determine ageing and the final toughness of meat.

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