MEAT TENDERIZATION BY ACIDIC LISOSOMAL PROTEINASES ASSOCIATED WITH GENOTYPE IN BEEF

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Background

Tenderness is one of the most important quality traits of beef for consumers. It is well known that meat tenderizes during storage, mainly due to the breakdown of key myofibrillar proteins, however the physicochemical and biochemical mechanisms involved in this process are still unclear. The calcium-dependent calpain system has been indicated as the major responsible for tenderness development in the early *post-mortem* periods (Koohmaraie, 1994). Nevertheless, lysosomal cathepsins may be involved in later tenderization processes, when pH conditions are favourable for acidic enzymes (Christensen et al., 2004). Ouali (1992) suggested that meat tenderization may result from the synergistic action of both calpains and cathepsins.

Objectives

The aim of the present study was to analyze the evolution of instrumental toughness and cathepsin B, B+L, D and H activities during later *post-mortem* storage of beef from different genotypes. Cytosolic and lysosomal enzymatic activities were separately considered, in order to obtain information of the differential contribution of both compartments to tenderization processes.

Materials and methods

Twenty yearling bulls of two local breeds from northern Spain, Asturiana de los Valles (AV) and Asturiana de la Montaña (AM) were studied. Animals of the AV breed were homozygous (*mh*/*mh*), heterozygous (*mh*/+) or normal (+/+) for muscular hypertrophy gene. Bulls were fattened by feeding concentrate meal and barley straw *ad libitum* and were slaughtered at a live weight around 500 kg.
At 24h *post-mortem* the ultimate pH (pH24) of the *Longissimus dorsi* muscle (LD) was measured at the 5th rib level. The muscle was sliced, vacuum packed, aged at 4º C and stored at -20ºC for further analysis. Samples for toughness and enzymatic analyses were aged 7, 14 and 21 days (except samples of double-muscled (*mh*/*mh*) AV bulls which were aged 3, 7 and 14 days). Water holding capacity was estimated with two different methods: drip loss at 48h *post-mortem* (Honikel, 1998) and expressible juice (EJ) at 7 days ageing (Sierra, 1973). Warner-Bratzler (WB) shear force (kg) was measured on cooked meat in an Instron 1011 equipment. Enzyme assays were performed on lysosomal and cytosolic extracts, obtained according to Béchet et al. (1986). The cysteine proteinases B (EC3.4.22.1), B+L (L; EC.3.4.22.15) and H (EC.3.4.22.16) were assayed fluorimetrically (Barret, 1980, modified by Schreus et al., 1995) using different specific substrates. The aspartate proteinase cathepsin D (EC3.4.23.5) was assayed spectrophotometrically (Takahashi & Tang, 1981) with modifications (Schreus et al., 1995). Protein concentration was determined as described by Bradford (1976). Enzyme activities were expressed as enzymatic units/mg of protein.
An ANOVA was used to analyze the effect of genotype on the physico-chemical and enzymatic variables. The effect of ageing period on each genotype was analyzed by ANOVA with ageing time and animal as fixed factors. Differences between means were test by LSD procedure. Principal component analysis was made to describe the relationships between physico-chemical and enzymatic variables at 7 and 14 days of ageing. All the statistical analyses were performed using the SPSS programme 11.5 (2002).

Results and discussion

The genotype affected to the ultimate pH ($p<0.05$), which was significantly lower in meat of AV bulls with muscular hypertrophy (*mh*/*mh* or *mh*/+) than in AM bulls. This could be result of the higher proportion of fast-twitch fibers and the higher glycolytic metabolism in the LD muscle of double-muscled animals (Oliván

et al., 2004). Genotype affected also significantly to the drip loss of meat at 48h *post-mortem* (p<0,001), with meat of *mh*/*mh* AV bulls showing higher juice losses than any other genotype.

Table 1. Effect of genotype on pH24 and water holding capacity of meat.

	GENOTYPE				
	AV (*mh*/*mh*)	**AV (*mh*/+)**	**AV (+/+)**	**AM(+/+)**	**Sign.**
N	5	5	5	5	
pH24	5.48 a	5.53 a	5.58 ab	5.67 b	*
Drip loss (%)	2.68 a	1.18 b	1.49 b	0.81 b	***
EJ (%)	21.72 a	20.75 ab	20.35 ab	19.15 b	NS

Means in the same row followed by different letters are significantly different (p<0.05).

Figure 1(a) shows the tenderization pattern of the different genotypes during the ageing process. Meat of AV bulls homozygous (*mh*/*mh*) or heterozygous (*mh*/+) for muscular hypertrophy showed a higher tenderization rate than AV (+/+) or AM (+/+) bulls. This produced significant differences among genotypes in the WB shear force after long storage (14 and 21 days, Table 2), when meat of AM breed showed the highest toughness. This higher tenderization rate of meat from double-muscled bulls may be related with the lower ultimate pH recorded at 24h *post-mortem* and its positive influence on the activity of acidic enzymes, like cathepsins.

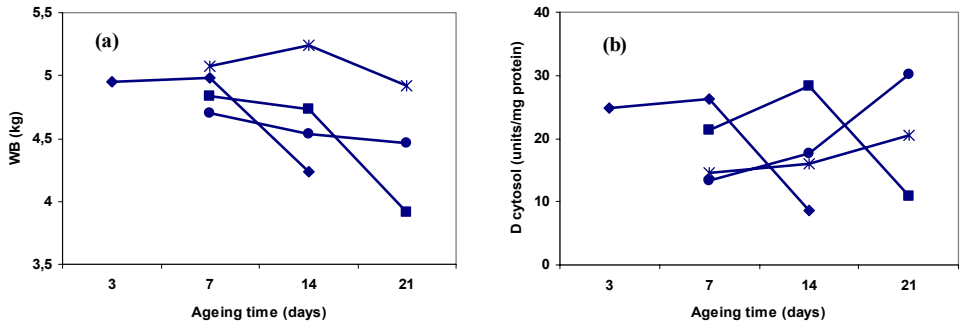

Figure 1. Time course changes in (a) WB shear force and (b) D cathepsin activity in cytosol extracts of the different genotypes (♦: *mh*/*mh* AV; ■: *mh*/+ AV, ●: +/+ AV; *: AM).

The evolution of the activity of cathepsins showed a similar pattern in lysosome and cytosolic domains, since they are complementary processes: the release of lysosomal proteases to cytosol and consistent breakdown of proteins by them produces an increase of endosomal vesicles, which interact to lysosomes giving them new substrates to act on (Alberts et al., 2002). In general enzyme activity levels were higher in cytosolic than in lysosomal extracts in all periods (Table 2), which could mean that the leakage of enzymes from lysosomes started in an early stage. The activity of cathepsins B, B+L, D and H at 7 days *post-mortem* was higher in meat of AV bulls with muscular hypertrophy (*mh*/*mh*, *mh*/+) than in other genotypes (AV(+/+), AM) in both extracts, but only reached significant differences in cytosolic space (for H cathepsin also in lysosomes). These data contribute to the hypothesis of faster tenderization rate of meat from double-muscled bulls, probably due to the lower pH conditions detected on the LD at early *post-mortem* periods, which activate processes of cellular membrane breakdown and enhance the release of the lysosomal proteases to the cytosol, as proposed by Berge et al. (2001). At later ageing the activity of most cathepsins (B, B+L and H) decreased both in lysosomal and cytosolic compartiments, probably due to the drop of availability of specific substrates, being this decrease more pronounced in double-muscled bulls than in other genotypes.

Cathepsin D showed the highest activity in both extracts and cathepsin H the lowest. That increase of cathepsin D along *post-mortem* ageing seems to be consequence of the absence of an endogenous inhibitor in muscular tissue specific for this enzyme. Rather, the role of cathepsin D as regulator of cystein-proteases could be responsible of the low activity of B, L and H cathepsins observed (Lenarcic et al., 1991), although it has been described that cystatin activity in the LD muscle is low (Koohmarie et al., 1988).

The activity of the D isoform in the cytosol showed different pattern in double-muscled (*mh*/*mh* and *mh*/+) and normal (AV(+/+) and AM) bulls (Fig. 1b). In animals with muscular hypertrophy the activity of cathepsin D was high at early and medium storage times (3-7 or 7-14 days, respectively) and showed a strong decrease after long storage (14 and 21 days respectively). However, in AV (+/+) and AM bulls there was a continuous increase of cathepsin D activity in the cytosol along the ageing period studied, which could

indicate an important implication of cathepsin D at the end of the tenderization process, that seems to be reached at longer ageing periods than those studied for these genotypes.
Figure 2 shows the relationship between the different parameters obtained through principal component (PC) analysis. Two groups of variables were clearly distinguished on the first PC. The first group included juice losses (drip loss and EJ) and high activity of all cathepsins (H, D, B and B+L) in the cytosol and in lysosomes at 7 days *post-mortem* This means that meat with high proteolytic activity at 7 days ageing exhibited higher juice losses, as a consequence of miofibrilar structure destabilization due to destruction of sarcomeres and fragmentation of actin-miosin complex. These variables were negatively correlated with the other group, composed by toughness (WB) at 14 days *post-mortem* and high enzyme activities in lysosomes (B, B+L H, D,) and cytosol (B, B+L, D) at 14 days.

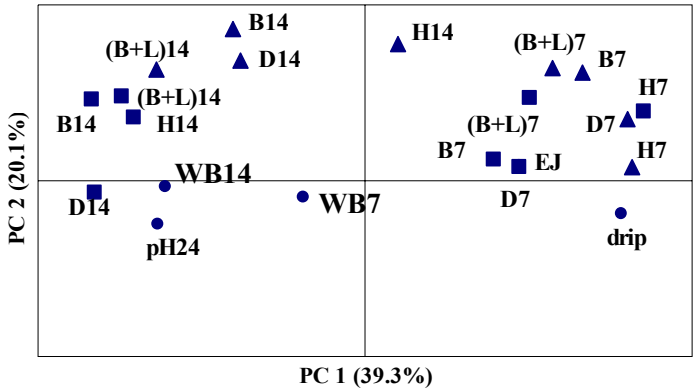

Figure 2. Principal component analysis of physico-chemical traits (●) and enzyme activities (■: in lysosome, ▲: in cytosol) at 7 and 14 days post-mortem for the whole set of samples.

Conclusions

The evolution of instrumental toughness and cathepsin activity in cytosolic and lysosomal extracts showed different pattern in the different genotypes studied. Homozygous or heterozygous animals for muscular hypertrophy presented higher enzymatic activity at early ageing periods and a higher tenderization rate than normal AV (+/+) or AM (+/+) bulls, for which the end of the tenderization process should be expected at longer ageing times. It seems that cathepsin D is the most implicated in later tenderization processes.

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Table 2. Effect of genotype on WB shear force and enzyme activities and evolution of each variable during the ageing period.

	GENOTYPE				
	AV (*mh*/*mh*)	**AV (*mh*/+)**	**AV (+/+)**	**AM(+/+)**	**Sign.**
WB shear force (kg):					
WB – 3 days	4.95 A				
WB – 7 days	4.98 A	4.84 A	4.70	5.08	NS
WB – 14 days	4.24_{a} B	4.73_{ab} AB	4.54_{a}	5.24_{b}	*
WB – 21 days		3.91_{a} B	4.46_{ab}	4.92_{b}	NS
Sign.	*	NS	NS	NS	
Enzyme activity in lysosome extracts (units/mg protein):					
B – 3 days	0.83 A				
B – 7 days	0.88 A	0.86 A	0.88 A	0.76	NS
B – 14 days	0.34_{a} B	0.85_{b} A	0.69_{c} B	0.88_{b}	***
B – 21 days		0.41_{a} B	0.72_{b} AB	0.74_{b}	***
Sign.	***	***	NS	NS	
(B+L) – 3 days	0.83 A				
(B+L) – 7 days	1.21_{a} B	1.20_{a} A	0.75_{b}	1.05_{ab}	NS
(B+L) – 14 days	0.40_{a} C	1.25_{b} A	0.91_{b}	1.26_{b}	***
(B+L) – 21 days		0.44_{a} B	1.01_{b}	0.66_{a}	**
Sign.	***	***	NS	NS	
H – 3 days	0.32 A				
H – 7 days	0.45_{a} B	0.38_{b} A	0.22_{c}	0.22_{c} A	***
H – 14 days	0.14_{a} C	0.41_{b} A	0.34_{b}	0.38_{b} B	***
H – 21 days		0.16_{a} B	0.30_{b}	0.29_{b} AB	**
Sign.	***	**	NS	*	
D – 3 days	5.62 A				
D – 7 days	4.36 A	3.24 AB	3.09 A	3.53 A	NS
D – 14 days	1.60_{a} B	3.62_{b} A	5.22_{c} B	7.41_{d} B	***
D – 21 days		2.44_{a} B	2.89_{a} A	4.58_{b} A	**
Sign.	*	NS	*	***	
Enzyme activity in cytosol extracts(units/mg protein):					
B – 3 days	1.30 A				
B – 7 days	1.34_{a} A	1.38_{a} A	0.68_{b} A	0.78_{b} A	***
B – 14 days	0.60_{a} B	1.64_{c} A	0.84_{ab} AB	1.14_{b} B	***
B – 21 days		0.85_{a} B	1.01_{b} B	1.06_{b} B	*
Sign.	***	**	*	*	
(B+L) – 3 days	1.13 AB				
(B+L) – 7 days	1.49_{ac} A	1.56_{a} A	0.75_{b} A	1.08_{bc} A	**
(B+L) – 14 days	0.81_{a} B	2.07_{b} B	1.27_{a} B	1.88_{b} B	***
(B+L) – 21 days		0.95 C	1.34 B	1.26 AB	NS
Sign.	*	**	*	NS	
H – 3 days	0.32 A				
H – 7 days	0.51_{c} B	0.36_{b} A	0.25_{ab} A	0.21_{a} A	***
H – 14 days	0.15_{a} C	0.32_{b} AB	0.08_{a} B	0.12_{a} B	**
H – 21 days		0.19_{a} B	0.37_{b} C	0.35_{b} C	***
Sign.	***	NS	***	***	
D – 3 days	24.74 A				
D – 7 days	26.30_{a} A	21.85_{b} A	13.32_{c} A	14.63_{c} A	***
D – 14 days	8.52_{a} B	24.91_{b} A	17.61_{c} A	15.93_{c} A	***
D – 21 days		10.85_{a} B	30.11_{b} B	20.54_{c} B	***
Sign.	***	***	***	*	

Means in the same row followed by different subscripts are significantly different ($p<0.05$).
For a given genotype, values in the same column with different capital letters are significantly different ($p<0.05$).