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MEAT TOUGHENING DURING AGEING ?

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Introduction

Post mortem (pm) storage of bovine carcasses at refrigeration temperatures is known to improve meat tenderness (Goll et al., 1964). The results of previous research, in wich we injected several exogenous protease effectors in cylindrical bore samples at 1 day pm, suggest a dominant role of cysteine proteinase activity and mainly calpains in ageing (Uytterhaegen et al., 1994). However the results obtained in this study showed a remarkable finding: the samples injected with a cysteine proteinase inhibitor were significantly tougher ($p<0.05$) than the control samples (not injected) frozen at 1 day pm (table 1). The present article proposes a hypothesis trying to explain this 'toughening effect'.

Table 1: Warner Bratzler shear force (WBS) values of cylindrical cork bore samples injected at 1 day pm with a cysteine proteinase inhibitor and aged for 7 more days (WBS day 8) compared with WBS values of non injected samples, frozen at 1 day pm. Values were taken from Uytterhaegen et al.(1994).

Inhibitor injected	WBS day 1 (N)	WBS day 8 (N)	p value
Leupeptine	53.2	62.7	0.004
Calpain inhibitor I	53.2	66.3	0.000
E64	61.7	75.0	0.001
E64	59.3	65.1	0.131
Calpain inhibitor I	50.6	59.7	0.005

Material and methods

All meat samples used were steaks of longissimus thoracis (LT) taken 1 day pm adjacent to the 7th thoracic rib of either Belgian White Red bulls (1 year old, ±500 kg live weight) or double muscled Belgian Blue White bulls (18 months old, ±700 kg live weight). Of each steak, about 30 cylindrical cork bore samples (diameter 1.27 cm) were taken parallel to the fiber direction and randomly alotted to two groups. The injection was done with a microsyringue (about 10% vol/w).

Warner Bratzler shear force (WBS) measurements, myofibrillar protein salt solubility and semi-quantitative sodium dodecyl sulphate polyacrylamide gelelectrophoresis (SDS-PAGE) were carried out as discribed before (Claeys et al., 1994, 1995)

Experiments, results and discussion

First, we investigated if the injection itself of a solvent in meat samples has an effect on WBS-measurements. Therefore, we compared WBS values of samples injected with distilled water with non-injected ones, determined before and after ageing. Results did not show significant ($p<0.05$) differences. Another possible explanation is that some proteolysis and tenderization occured in the (non-injected) 1 day pm samples upon heating (before WBS determination), what would not be possible in the samples injected with inhibitor. WBS values, determined 1 hour after injection (allowing distribution) of 2 mM calpain inhibitor I (Boehringer Mannheim 1086 090) in LT muscle bore samples (Belgian Blue White bulls), were not different ($p<0.05$) from the non-injected control samples (test carried out on 5 different LT steaks). So, tenderization through cysteine proteinase activation upon heating is negligable.

To investigate if this toughening effect in cysteine proteinase inhibitor injected samples gradually increases with time pm, all cork bore samples of an LT steak (Belgian White Red bull, 24 h pm) were injected with a mixture of 1.6 mM calpain inhibitor I and 0.3 mM E64 (Fluka Chemie AG nr 45370) and randomly alotted in 4 groups. One group was immediately frozen at -80 °C, whereas the others were stored at 2°C up to 3, 5 or 8 days pm and also frozen at -80°C. Half of the samples of each group were used for WBS determination and cooking losses; the rest was used for SDS-PAGE and total crude protein determination. Results (table 2) show that WBS increased significantly ($p=0.005$) with time pm This means that in beef in which all cysteine proteinase activity is inhibited, a processus takes place which continuously toughens the meat. The fact that cooking losses decrease and % crude protein (on fresh material) increases with time pm indicated that the injected solution gradually comes out of the meat with time pm.

Table 2: Warner Bratzler shear force values (WBS), cooking losses and crude protein determination values (fresh material) of cystein proteinase injected samples (1 day pm) at different times of storage at 2°C.

Day pm	WBS (N)	cooking losses (%)	% crude protein
1	44.7	37.4	18.3
3	49.1	37.3	18.6
5	49.8	36.5	19.5
8	53.1	35.9	20.4

The degree by which the cysteine proteinase activity was inhibited can be deduced from the result of semi-quantitative SDS-PAGE (table 3). Troponin T concentrations still decrease slightly between 1 and 3 days pm, but no further breakdown could be detected, suggesting that

complete inhibition took some time, although this is not confirmed by a parallel increase of 30 kD component. Post mortem proteolysis however was very limited, confirmed by the lack of breakdown of titin and nebulin. Both proteins even increased significantly ($p<0.05$) with time pm The reason for this is not clear. The increase of the 34 kDalton protein is explained by the assumption that this protein is of sarcoplasmic origin and its presence in the myofibrillar fraction is a result of denaturation, increasing with time pm

Table 3: Protein concentrations as derived from SDS-PAGE (mg BSA-equivalents/g myofibrillar crude protein) of cysteine proteinase inhibitor injected longissimus thoracis samples at 4 different times pm

Protein	Day 1	Day 3	Day 5	Day 8	p value
Titin	46.5^{a}	45.8^{a}	50.7^{b}	57.9^{c}	0.00
Nebulin	40.2^{a}	42.7^{a}	38.9^{a}	47.5^{b}	0.02
Filamin	2.4	2.5	2.4	3.4	0.85
Creatin Phospho Kinase	20.6	20.3	21.5	22.3	0.74
Troponin T	11.2^{a}	8.7^{b}	7.8^{b}	7.9^{b}	0.01
Troponin T2	7.3^{a}	5.9^{b}	4.8^{b}	5.1^{b}	0.02
34 kDalton	5.6^{a}	9.2^{b}	10.0^{b}	15.9^{c}	0.00
30 kDalton	5.0	4.1	4.4	4.5	0.78

$^{a, b, c}$: different superscripts within a row mean significant ($p<0.05$) different values.

We suggest the following explanation for the toughening effect evident from the data shown in table 1.
It is known that, starting from the moment of slaughter, the conversion of muscle to meat is characterised by a total exhaustion of the energy supplying compounds (especially ATP) with a parallel pH drop leading to a phenomenon called rigor mortis (Ouali et al, 1992). This situation is characterised by an attachment of cross-bridges between thick and thin filaments and leads to a large increase of the toughness of meat (Offer and Knight, 1988; Ouali et al., 1992). The pH drop normally continues to a final value of about 5.5 within 24 hours. This value is close to the iso-electric pH of the myofibrillar proteins with a loss of water as a consequence (finally resulting in drip losses) and a decrease of the lateral electrostatic repulse forces between the filaments (Offer and Knight, 1988). All this is accompanied by a shrinkage of the myofibrils, leaving gaps between fiber bundels and also between fibers and the endomysial network (Swatland and Belfry, 1985; Offer and Knight, 1988). Further, during rigor mortis onset, the ionic strenght in muscle (and thus the osmotic pressure) increases (Ouali, 1992), leading to a further decrease of the electrostatic repulsion forces of the filaments (Offer and Knight, 1988). Our hypothesis is that during ageing of beef, the repulsion forces between filaments become sufficiently low for hydrophobic interactions to take place between these filaments. These non covalent bonds between hydrophobic parts of different filaments would result in a more extensive cohesion and a larger force needed to tear muscle fibers apart with a higher shear force as a consequence. If this hypothesis is correct, one should expect that in cystein proteinase inhibitor injected samples the solubility of the myofibrillar proteins will decrease with time pm instead of an increase as is normally observed (Ouali, 1992). We have tested this by measuring the myofibrillar salt solubility (MPS) at both pH 5.5 and 7 of the samples from the above mentioned experiment. The results (table 4) show indeed a significant ($p=0.03$) decrease of MPS at pH 7 while the decrease at pH 5.5 was less pronounced ($p=0.06$). Separation of these dissolved proteins showed that the solubility of actin decreased more pronounced than that of myosin both at pH 5.5 and 7 (results not shown). Because denaturation of myosin only occurs during the pm pH drop (Offer and Knight, 1988), we suppose that the MPS decrease measured is not due to a further denaturation of these proteins. We submit these findings as support for the proposed hypothesis, but further research on this interesting phenomenon is certainly needed.

Table 4: Myofibrillar protein salt solubility at pH 5.5 and 7 expressed as mg soluble protein/g total crude protein at different times pm of samples injected with a cystein proteinase inhibitor.

	Day 1	Day 3	Day 5	Day 8	p value
pH 5.5	72.1	70.3	66.1	65.5	0.062
pH 7	138.7	134.6	132.8	122.1	0.029

Conclusion

The preliminary results in the presented paper show that beef, in which all cystein proteinase activity is blocked by injecting an inhibitor, toughens upon storage at refrigeration temperatures. The hypothesis proposed, for explaining this phenomenon should however be subjected to further investigation.

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