DESMIN, 30KDA POST-MORTEM PATTERNS AND THE CALPAIN SYSTEM AS INDICATORS OF TENDERNESS IN BEEF ANIMALS

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Introduction

One of the most important meat quality attributes is tenderness (Ouali, 1991). Techniques such as sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western-blotting could be used for assessing meat tenderness. The degradation of proteins such as titin, nebulin, and desmin have been shown to play a role in the development in meat tenderness (Huff-Lonergan *et al.* 1996) and act as substrates for the calpain system known to be involved with the *post mortem* ageing of meat. These techniques are gradually becoming more sensitive, rapid and reliable as technologies develop in the form of capillary electrophoresis combined with mass spectroscopy. The purposes of this paper is to illustrate the correlation between the degradation patterns of desmin and the development of the 30kDa protein on the individual level and correlate them with different aspects of the calpain system and to determine how the data corresponds to the measured shear force on the *M. longissimus*.

Materials and Methods

The four animals (Brahman-144; Nguni-39; Simmental-130; Nguni-49) chosen as examples for this article form part of a larger project as described by Strydom and Frylinck (2005). These carcasses were not electrically stimulated (NS) as electrical stimulation (ES) influences the processes of meat tenderness. This paper focussed on the expression of the inherent tenderness characteristics without external *post mortem* influences. To study the degradation of desmin and appearance of the ~30 kDa degradation products as a result of tenderisation the *M. longissimus* proteins from samples taken at 1, 7, and 14 days *post mortem,* were separated using 30%T 0.5%C SDS-PAGE separation gels with 12%T, 12.5%C stacking gels (Fritz *et al.*, 1989). The separated muscle protein bands by means of SDS-PAGE electrophoresis were analysed by densitometry with the help a video image analyser equipped with the ImageMaster 1D Software (Amersham Pharmacia Biotech). The transfer and Western blotting procedures were carried out in accordance with the procedure of Huff-Lonergan (1996). The electrophoresis and Western immuno-blot patterns were analysed densitometrically with the ImageMaster 1D Software (Amersham Pharmacia Biotech). Calpains and calpastatin were extracted from frozen samples (Dransfield 1996) and separated by means of a two-step gradient ion-exchange chromatography-method (Geesink and Koohmaraie 1999). Calpain assays were done using azo-casein as substrate (Dransfield 1996) and one unit of calpain activity was defined as an increase in absorbance at 366 nm of 1.0 per hour at 25 °C. One unit of calpastatin activity was defined as the amount that inhibited one unit of m-calpain activity. Myofibril fragment lengths were measured using a Video Image Analyzer and tenderness was measured by Warner Bratzler shear force (SF) measurements (Strydom and Frylinck, 2005).

Results and Discussion

The intensities of the diminishing desmin protein (Figure 1) and the developing intensities of the 30 kDa protein bands as revealed by Western immuno-blotting and SDS-PAGE techniques, at 1-, 7-, and 14d *post mortem* in Brahman-144, Nguni-39, Simmental-130, and Nguni-49 are shown in Table 1. High correlations existed between these proteins and the calpain system analysed by means of the myofibril fragmentation at 1 day (r=0.68 to 0.92 for desmin; r=-0.71 to -0.81 for 30 kDa), 7 d *post mortem* (r=0.70 to 0.91 for desmin; r=-0.50 to -0.80 for 30 kDa), and rate of ageing (MFL at 1 d p.m – MFL at 14 d p.m) (r=0.68 to 0.92 for desmin; r=-0.70 to -0.85 for 30 kDa). Calpastatin and μ-calpain activity levels measured at 1 h post slaughter as well as the calpastatin/μ-calpain ratio (Table 2) correlated with rate of desmin degradation (r=0.80, –0.55, and 0.76 respectively) and 30 kDa formation (r=-0.83, 0.65, and -0.65 respectively). In contrast to these results, SF only correlated with diminishing desmin and formation of 30 kDa in respect with the tempo of tenderisation (SF at 1 d p.m. – SF at 14 d p.m.) (r=0.86 and 0.84 respectively). The actual shear force measured at 1 d and 14 d did not correlate well with the intensities of these proteins. The Br-144 and Sm-130 animals (Table 2) were the tougher of the four animal, but the MFL at 14 d p.m. were similar for al the animals. Also, the calpastatin level of Br-144 was unexpectedly the most favourable of the four animals. This show that one should judge all animals on an individual level, and that the variation between animals within breeds must be kept in mind.

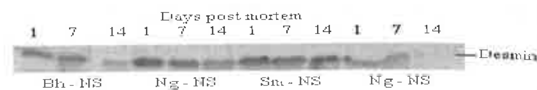

Figure 1: Immuno-blots showing the degradation of desmin over time at 1, 7, and 14 days *post mortem* in *M. longissimus* of the Brahman (Bh-144), Nguni (Ng-39), Simmental (Sm-130) and Nguni (Ng-49).

Table 1: Densitometric scans of SDS-PAGE and Western blots of nebulin, desmin and the 30 kDa degradation protein (µg/µl) of examples of four animals representing three genotypes; Brahman-X, Nguni and Simmental-X.

Protein	Br-144	Ng-39	Sm-130	Ng-49
• 30 kDa (SDS-PAGE)				
- 1 d p.m.	0.028	0.017	0.011	0.000
- 7 d p.m.	0.062	0.019	0.018	0.039
- 14 d p.m.	0.153	0.052	0.059	0.138
- (1-14) d p.m.	0.125	0.035	0.048	0.138
• Desmin (Western blot)				
- 1 d p.m.	8.339	9.896	10.185	8.512
- 7 d p.m.	7.417	9.147	8.743	7.936
- 14 d p.m.	5.571	8.051	8.685	7.878
- (1-14) d p.m.	2.768	1.845	1.500	0.634

Table 2: Tenderness (SF) and calpain system measurements on examples of M. longissimus of four individual animals representing three genotypes; Brahman-X, Nguni, and Simmental-X.

Characteristic	Br-144	Ng-39	Sm-130	Ng-49
SF – 1 d p.m. (kg)	10.48	6.61	9.54	6.25
SF – 14 d p.m. (kg)	6.17	4.08	7.30	4.33
MFL – 7 d p.m. (µm)	25.9	34.0	41.3	31.8
MFL – 14 d p.m. (µm)	23.1	23.2	24.5	21.8
MFL – (1 – 14) d p.m. (µm)	9.48	26.8	37.5	16.8
Calpastatin (U/g meat)	2.13	2.12	3.13	3.66
µ-Calpain (U/g meat)	1.26	1.21	1.16	2.11
Calpastatin/µ-calpain	2.00	2.24	2.76	1.74

SF = Warner Bratzler shear force; MFL = myofibril fragment length; d p.m. – days *post mortem*

Conclusions

The tenderisation of meat is a multi-factorial event, therefore it will not be enough to only measure the contribution of the tenderisation mechanism involving the calpain system on the individual animal level in order to predict its potential meat tenderness. New technologies must include the potential contribution of the other mechanisms, such as the level of contraction of actin and myosin, as well as the contribution of the connective tissue characteristics.

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