THE INFLUENCE OF DIFFERENT MODERATE COOLING CONDITIONS ON TENDERNESS AND TROPONIN-T-, 30 KDa-, AND TITIN-CONCENTRATIONS OF BULL LONGISSIMUS DORSI.

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INTRODUCTION

We have previously observed a considerable variability in tenderness of young bull longissimus dorsi (LD) under very moderate cooling conditions (Buts et al., 1984, 1986a).
An important part of this variability could be explained by differences in Troponin-T and 30 KDa concentrations determined after separation of the myofibrillar proteins on SDS-Page (Buts et al., 1986b) indicating differences in extent of post-mortal proteolysis as a main cause of tenderness variability.
Maintaining a high temperature early post mortem may result in improved tenderness (e.g. Lochner et al., 1980; Marsh et al, 1981, 1983; Petäjä et al., 1985) probably by enhancement of activity of proteolytic enzymes. On the other hand Tornberg and Larsson (1986) found it is more beneficial to allow meat to go into rigor at 15°C than at 37°C, because the effect of slow rigor development and minimal contraction at 15°C is more important for tenderness of aged beef than the hypothetical effect of increased release of proteolytic enzymes at 37°C.
From this it is clear that temperature during onset of rigor may influence meat tenderness apart from prevention of cold-shortening as it may determine both rate of shortening at rigor and of post mortal proteolysis.
In two experiments temperature during the onset of rigor was varied in different ways in order to study the relation between tenderness and temperature during this important period.

MATERIALS AND METHODS

Animals and treatment of carcasses:
All animals used are from the progeny testing station RSC (B-9258 Scheldewindeke, Belgium). In the first experiment 10 one-year old bulls (mean values ± standard error for live weight: 461 ± 25 kg and dressing-%: 59.4 ± 1.4 %, were slaughtered in the slaughterhouse of our laboratory after captive bold stunning and pithing. Carcass halves were transferred to a cooling room at 4 °C, 0.5 m/sec, one hour p.m. Cooling of right carcass halves was interrupted by moving them to a room at 13°C, 0 m/sec after 3 to 6 h post mortem (p.m.) until 12 h p.m. when they are placed back with the control halves. In the second experiment two groups of animals of 10 and 8 animals respectively, (mean values ± standard error for live weight: 466 ± 45 and 477 ± 34 kg and dressing-%: 57.3 ± 2.1 and 58.8 ± 2.1 % respectively) were slaughtered on different days (one in december and one in april). Control carcass halves (left and right alternating) were cooled at 4°C, 1 m/sec while the other halves were cooled at 13°C, 0.5 m/sec until 8 h p.m. then cooling-room temperature was set to 4°C.
Temperature was measured 7.5 cm deep in the centre of the Longissimus dorsi (8th thoracic rib) either with a portable temperature meter (Technotherm 9503, Instrulab, Brussels, Belgium) or with Pt 100 thermocouples (1/3 Din, Degussa, Brussels, Belgium) connected to a Honeywell recorder outside the cooling room. pH was measured with an Ingold combination electrode (Weilheim, BRD) attached to a digital pH meter (Knick Portamess 651, Knick, Berlin, BRD) at regular intervals post mortem.
In both experiments the LD (8th thoracic rib) was removed ca 24 h p.m. and after trimming of

fat, samples were vacuum packed in polyamide laminated polyethylene (Sidamyl-X, typ EAK 41, Sidac, Ghent, Belgium) and further conditioned at 4°C until 8 days p.m. Then samples were frozen and further preserved at - 18°C for 2 to 6 weeks (exp^{t} 1) or 2 to 3 months (exp^{t} 2). After removal from the deep-freezer cuts were thawed in a standardized way (1 day at 4°C refrigerator and ½ h waterbath at 20°C).

Determinations:
After removal of the cuts from the vacuum bag, drip loss was determined and a subsample of about 50 g covering the whole cutting surface of the sample was removed for isolation of the myofibrillar proteins and subsequent separation on Polyacrylamide gels and determination of sarcomere length (SL). Remaining cuts were then heated in open plastic bags by immersion (1 hour) in a waterbath at 75°C and subsequently cooled under running tap water (ca 10 minutes) and cooking loss determined. Then 20 to 30 cork bore samples (diam. 1.27 cm) were taken parallel to the fibre direction. In exp^{t} 1, 16 samples were used for tenderness evaluation by an eight member trained taste Panel and the remaining samples for the determination of the Warner Bratzler Shear force (WBS) while in exp^{t} 2 all bore samples were used for determination of WBS.

Methods:
Bag drip was measured by the weight difference before and after vacuum packing (sample wiped with a cloth before weighing) and expressed as percentage of initial weight.
Sarcomere length was measured on fresh subsamples of about 10 g fixed in 2.5% glutaraldehyd using laser diffraction as described by Vandendriessche et al. (1984). Average values of measurements of 80 different fibres were obtainded.
Myofibrillar proteins were isolated according to the procedure of Parrish et al. (1973) and isolated myofibrills were dissolved overnight (room temperature, magnetic stirring) in imidazole buffer (0.01 M, pH 7.0) containig 2% SDS and 2% 2-mercaptoethanol. Further preparation of the isolated myofibrils and subsequent electrophoresis was performed as described in Buts et al. (1986b) and Claeys et al. (1989)
After electrophoresis, gels (8 and 4.6 % total acrylamide concentration, 3.1 % crosslinking) were stained with Coomassie Brillant blue and after destaining scanned using a Beckmann model R-112 densitometer. Peak areas were determined and protein concentrations (expressed as BSA equivalents) calculated.
Cooking loss was determined by weight difference before and after heating and expressed as percentage of weigth before cooking.
Warner-Bratzler peak Shear force was determined with a WB-shear mounted on an Instron 1140 Food tester (Instron Ltd., High Wycombe) perpendicular to the fibre direction
Taste panel tenderness rating (exp^{t} 1 only) were obtained using a scale with 8 subdivisions (1 = very tender, ..., 8= very though) as described by Demeyer et al. (1983). The ratings of eight members were averaged.

RESULTS
In table 1 temperature and pH results are shown. For rate of cooling regressions following $Y = a + b\,(1 - e^{-ct})$ (y = muscle temperature °C and t = time post-mortem) were calculated. In this model **a** is the begin temperature at time 0 (theoretical, because the lag time for the installation of the temperature gradient is not taken into acount), **b** the difference between muscle and cooling room temperature at zero time and **c** the rate constant of cooling. The cooling rate at each time post mortem (within the exponential phase of cooling) can be calculated as follows: $dY/dt = bc\,e^{-ct}$ (°C/h). Decimal reduction time (time to accomplish 90% of cooling) can be calcultated as $t_{dec} = \ln(0.1)/-c$, analogous to the prodedure described by Moerman (1973). Decimal reduction time is also expressed on

Table 1: Mean values for temperature, cooling rate characteristics and pH.

	expt 1		expt 2			
			group 1		group 2	
	contr. (n=10)	treatm.	contr. (n=10)	treatm.	contr. (n=8)	treatm.
Temperature:						
1 h	39.0	38.6	32.0	38.9	39.8	39.9
2½ h	33.3	32.9	32.7	33.2	33.6	34.5
4 h	27.1	26.3	25.9	27.6*	27.2	29.5*
6 h	21.3	21.1	19.1	22.6*	21.7	25.4*
8 h	17.2	18.5*	14.6	20.1*	17.4	22.0*
10 h	12.3	15.2*	----	----	----	----
24 h	6.1	6.4	4.9	9.2*	5.5	9.4*
c[1]	0.146	----	0.164	0.145*	0.144	0.117*
t_{dec}[1]	15.8	----	14.1	16.1*	16.3	19.9*
t_{dec}/kg[1]	0.116	----	0.106	0.121*	0.116	0.142*
$t_{10°C}$[2]	14.7	17.4*	11.8	21.1*	13.7	22.0*
pH:						
1 h	6.80	6.82	6.97	6.98	6.79	6.76
2½ h	6.42	6.52	6.48	6.44	6.24	6.05
4 h	6.23	6.16	6.12	6.10	5.87	5.72
6 h	6.12	6.14	5.90	5.85	5.68	5.59*
10 h	5.57	5.68*	----	----	----	----
24 h	----	----	5.60	5.61	5.67	5.64*

level of significance: * at least $P<0.05$ (paired t-test)
[1]: c = rate constant of cooling (1/h); t_{dec}:decimal reduction time (h); t_{dec}/kg: decimal reduction time on carcass halve weight (h/kg).
[2]: time to reach 10 °C (h)

Table 2: Mean values for meat quality characteristics and protein fragmentation

	expt 1		expt 2			
			group 1		group 2	
	contr. (n=10)	treat.	contr. (n=10)	treat.	contr. (n=8)	treat.
Drip-loss (%)[1]	9.9	9.9	6.6	7.5	9.6	9.8
Cook.-loss (%)[1]	26.3	27.4	25.2	25.8	24.7	25.5
SL (µm)	1.87	1.83	1.87	1.89	1.99	1.98
WBS (N)	41.8	44.5	37.7^{a}	33.2^{b}	29.1	30.8
TP score	3.8	3.8	---	---	---	---
Myofibrillar proteins[2]:						
Titin	15.1	15.7	26.6	25.9	27.2	27.4
Troponin-T	7.6	7.1	3.8^{a}	1.4^{b}	1.0	1.5
30 kDa	10.3	11.1	10.9	12.6	15.4	16.1

[1]: For experiment 1, n=7.
[2]: concentration in µg BSA-equival. / mg myofibrillar protein
[a,b]: values with different superscript are significantly different ($p<0.05$; paired t-test) within experiments.
___: significantly different between experiments ($p<0.05$; t-test)

carcass half weight to correct for temperature differences due to differences in muscle weight. The model could not be applied in exp^t 1 because, due to the interruption of forced cooling, no exponential temperature decline was obtained.

From table 1 its clear that in experiment 1 the withdrawal of cooling resulted in higher muscle temperatures for treated halves during a period of time from 8 hour p.m. on, but this temperature difference has disappeared 24 h p.m.

In experiment 2 the difference in cooling conditions resulted in higher muscle temperatures in the treated halves from 4 h p.m. on, a difference that persisted up to 24 h p.m. All temperatures observed in group 1 (slaughtered in december) are lower than the corresponding values in group 2 (slaughtered in april). Furthermore faster rates of post-mortal glycolysis (pH) were observed group 2 as compared to group 1.

From table 2 it is clear that Mean Warner Bratzler Shear force (WBS) was not influenced by the treatment in the first experiment. For the first group of the second experiment mean WBS was lowered and protein fragmentation increased by the treatment. For the second group both control and treated halves were very tender. This was reflected in low Tn-T and high 30 KDa concentrations for both. Titin contents were not different within experiments but very different between expt 1 an experiment 2.

DISCUSSION

Results from exp^t 1 and exp^t 2 (group 1) suggest that a longer period of temperature difference in the early post mortal period is necessary for influence on tenderness, and that the action of temperature on tenderness is related to changes in proteolytic activity. For the second group of experiment 2 it seems that both control and treated halves were cooled slow enough resulting in optimal tenderness for both but there is probably also the effect of higher rate of post mortal glycolysis. The conditions for this group correspond remarkably well with those for which Marsh et al (1987) found the highest tenderness level (pH 3 h p.m ca 6 and corresponding temperature ca 27°C).

Sarcomere length as a measure of shortening of muscles is only very weakly correlated with WBS as is clear from the correlation matrix for all combined data shown in table 3. The significance of the correlation between WBS and SL is due to one (treated) halve of exp^t 1 with SL = 1.53 μ and WBS = 67.4 N, because without this halve the correlation coefficient (r) is -0.215 (not significant). The shorter sarcomere length for this halve clearly did not result from cold-shortening but may be due to so called "heat"- or "rigor"-shortening. The toughness however did clearly not only result from shortening because also the Tn-T and 30 KDa concentrations were relatively high and low (11.2 and 5.5 μg BSA-eq respectively) indicating relatively low post-mortal proteolytic degradation too.

It is clear that conditions for shortening at rigor are about optimal for the control animals. For the treated ones temperatures are somewhat higher (ca 20 °C at pH 5.8 - 6.0 for expt 1 and group 1 of expt 2 and near 30 °C for group 2 of expt 2) than the theoretical optimum of 15-20 °C. It must be mentioned here that temperature is measured in the warmest point of the LD so temperature is lower in the greatest part of the muscle. So it is obvious that, with one exception (see higher), shortening at rigor cannot explain the tenderness differences observed.

On the other hand it is clear from table 3 that an important part of tenderness variability can be explained by differences in proteolytic activity reflected in protein fragmentation. This confirms our earlier results (Buts et al 1986b; 1987) and the results of Seideman & Koohmaraie (1987) who could explain a great part of the variability in tenderness ratings (and shear force values) by differences in fragmentation index.

Table 3: Correlation matrix, mean, standard deviation and range for the poolded date for all halves (n=56).

	Tendern.[1]	WBS	SL	Titin	Tn-T	30KDa
Tendern.[1]	1.000	0.790**	-0.380	-0.245	0.732**	-0.766**
WBS		1.000	-0.338*	-0.030	0.759**	-0.732**
SL			1.000	0.451**	-0.114	0.227
Titin				1.000	-0.113	0.015
Tn-T					1.000	-0.814**
30KDa						1.000
Mean	3.8	36.6	1.90	25.6	3.9	12.5
Stand. Dev.	0.9	9.7	0.14	3.6	4.0	4.3
Min	2.6	20.9	1.53	17.2	0.2	4.4
Max	6.0	67.4	2.26	38.6	14.3	19.3

Level of significance: *: P<0.05; **: P<0.01
[1]: Tendern. = taste panel tenderness; exp[t] 1 only (n=20).

In figures 1 and 2 the relation between WBS and, respectively, Troponin-T and the 30 KDa component is shown. The relations found are not different from the ones we reported earlier (Buts et al, 1986b) for a similar group of animals. The introduction of SL as second independent variable in both relations allowed explanation of a significantly greater part of WBS variability than Tn-T alone (r^2=0.640) but could not explain more than 30 KDa alone.
For the relation between Tn-T and 30 KDa a similar regression equation as before (Buts et al., 1986b, 1987): $Tn\text{-}T_{conc}$ = 13.4 - 0.76 $30KDa_{conc}$ (r^2=0.663, s = 2.36) is found. This highly significant negative correlation may indicate that 30 KDa is a degradation product of Tn-T, but this correlation does not necessarily reflect a direct cause/effect relationship.
The relation between Titin and WBS is unclear and is influenced by the difference in titin concentrations between exp[t] 1 and the two other experiments for which we do not have an explanation. The fact that titin is influenced by a number of factors during storage of samples like time of frozen storage (Greaser et al, 1983) makes it difficult to indicate the contribution of titin in tenderness variability.

CONCLUSION:
An important part of tenderness variability can be explained by differences in (parallel) protein fragmentation (=differences in maturation), although the influence of the structure itself (reflected partly in sarcomere length) cannot be completely excluded even in the absence of cold-shortening.
It is also clear from our results that proteolytic activity can to some extent be influenced by modification of the cooling conditions during installation of rigor. A far better knowledge of the potential hydrolytic activity of proteinases, the activity of proteinase inhibitors and the tenderizing mechanism in general is needed before optimal cooling conditions for maximal tenderization can be indicated.

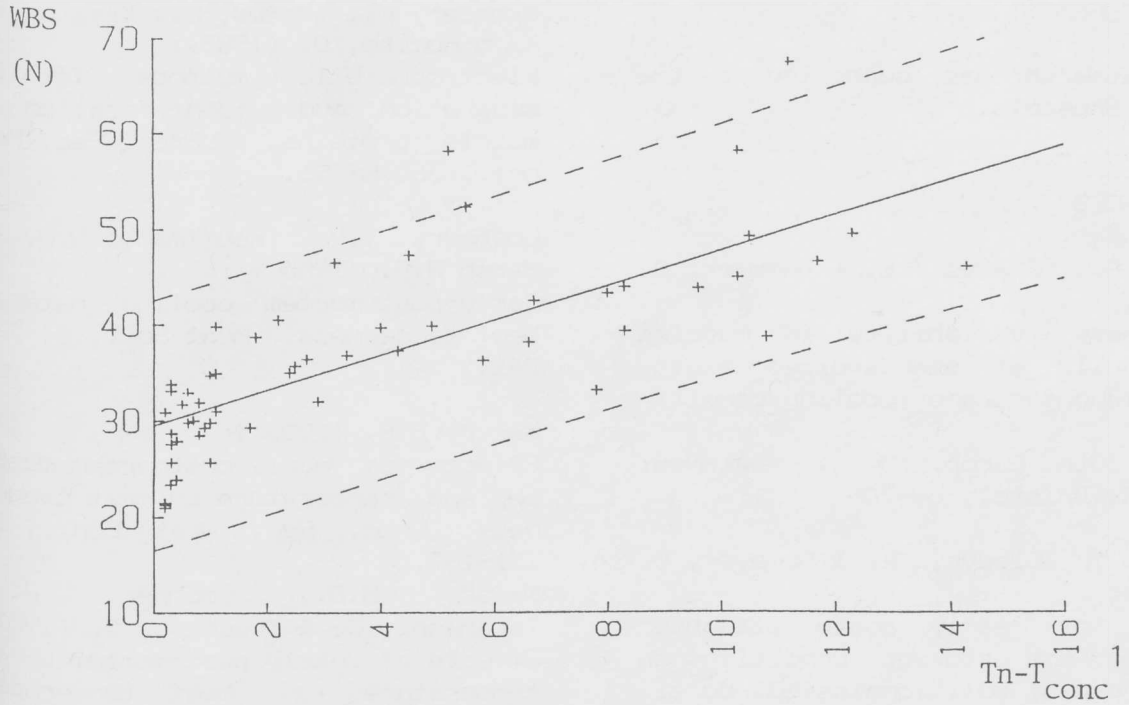

Figure 1 : Regression of WBS on Troponin-T concentr.
Regression equation : WBS = 29.5 + 1.83 $Tn\text{-}T_{conc}$
r^2 = 0.576; S = 6.38 (n = 56)

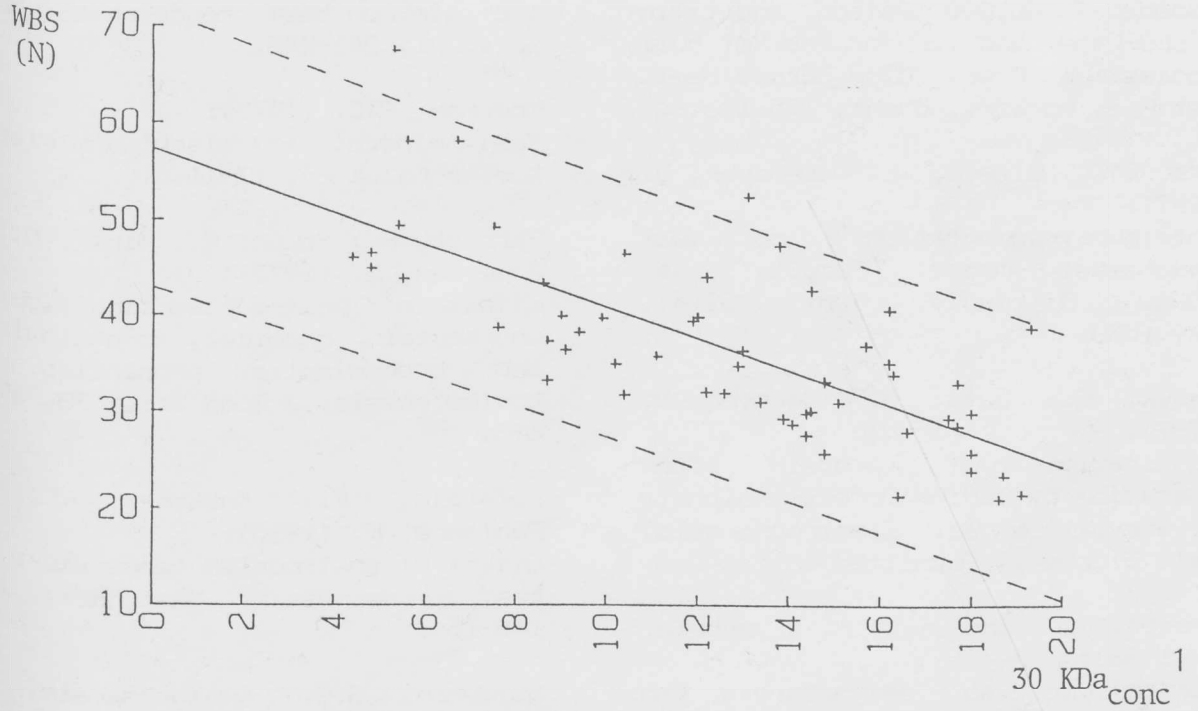

Figure 2 : Regression of WBS on 30 KDa concentr.
Regression equation : WBS = 57.2 - 1.64 $30\ KDa_{conc}$
r^2 = 0.537; S = 6.67 (n = 56)

[1] µg BSA-eq./mg myof. protein

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