Tenderness variability in muscles from bulls at standardized weight, age, slaughter and cooling conditions

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Introduction :

The effect of different animal or carcass characteristics or treatments on tenderness or meat quality has been the subject of many investigations and recently there is a markedly renewed attention for this subject as illustrated by the many recent publications in this area (e.g. Tatum et al., 1980; Wu et al., 1981; Mc Curdy et al., 1981; Koch et al., 1982; Tatum et al.,1982; Crouse et al., 1984).
The results of such investigations are often very different from each other and even contradictory as clearly illustrated by Bouton et al (1978) for the relation of animal age and tenderness. They noticed that results of investigations relating animal age on tenderness of meat could be divided into 3 groups: (a) results which showed a general decrease in tenderness with increased animal age; (b) results which showed no effect of age on tenderness and (c) results which showed an increase of tenderness with increased animal age. They therefore suggested that results of age/tenderness studies depend on age range, differences in carcass weight, muscle(s) chosen, the cooling rate of muscles and hence on chilling conditions, cooking methods and the method(s) used to asses the mechanical properties of the cooked muscle. This conclusion may probably be generalised for all investigations which try to relate animal or carcass characteristics or treatments to tenderness of meat. This is illustrated by a recent coordinated interlaboratory E.E.C. study reported by Dransfield et al. (1982). In this study it was concluded that although attempts to relate quality to different production factors were often confounded, differences in post-slaughter handling, particularly between producing countries, dominated eating quality. On the contrary breed, sex, age and fatness had relatively little influence on eating quality.
The above information leads to the conclusion that investigation of the influence of a single animal or carcass characteristic on meat tenderness seems to be possible only if all other factors influencing tenderness are held constant or, are at least recorded in order to quantitate their eventual effect.
Two experiments were done to find out if conditions could be standardized in such a way that the effect on tenderness is as constant as possible so that similar animals would give similar tenderness ratings. For reasons of standardization and ease of working, Warner-Bratzler peak Shear Force was chosen as (objective) tenderness measurement. In addition to this main investigation target, the relation between the Myofibrillar Fragmentation Index (MFI) determined on raw muscle and peak Shear Force (SF) determined on cooked muscles was studied.

Material and Methods

Animals and treatment of carcasses :

For the first experiment 26 bulls (from the progeny testing station RSC, B-9258 Scheldewindeke, Belgium) of two different breeds (Red and Red-White of Flanders)of exactly 1 year old and with a mean live weight (± SE) of 457 ± 6 kg and a mean dressing-% (± SE) of 64.5 ± 0.2 %, were slaughtered on 5 different days in the slaughterhouse of our laboratory. For the second experiment 34 bulls of the same origin as those of expt. 1 and with mean live weight (±SE) 487 ± 6 kg and dressing-% 59.0 ± 0.3, were slaughtered on 6 different days. In both experiments slaughtering was done after captive bolt stunning and pithing in a reproducible manner and the carcasses were transported to a cooling room (4 ± 2 °C) after exactly 68 ± 1 min. post-mortem (p.m.) (mean value ± SE) in experiment 1 and 64 ± 1 min. in expt. 2.
For both experiments temperature of two muscles of the right hand carcass halves (M. Longissimus dorsi 8th thoracic rib and M. Semitendinosus) was recorded continuously in a standardized way until 24 h p.m. as follows : Thermocouples (Pt 100, 1/3 DIN, Degussa) were inserted exactly 7.5 cm deep into the center of the Longissimus dorsi (LD) part corresponding to the 8th thoracic rib, and exactly 5 cm deep in the centre of the outside visible part of the Semitendinosus (ST). These thermocouples were connected with a Honeywell recorder outside the cooling room (accuracy: ± 0.15 °C) and each measuring point was monitored every 5 min from the moment the carcasses arrived in the cooling room up to 24 h p.m. The carcasses were placed every time in the same part of the same cooling room, this in order to achieve about the same cooling conditions for all carcasses.
The pH was measured with a portable pH meter (Knick portamess 651, Knick, Berlin, BRD) in both muscles at 1 h, 2.5 h, 4 h and 6 h p.m. at a depth of about 5 cm (each time mean value of 5 measurements). Between 24 and 28 h p.m. the LD 8th thoracic rib and the ST of the right carcass halves were removed, vacuum packed (for ST the middle part ± 3 cm thick was packed for experiment 1, and two adjacent parts A and B from the middle part of the muscle for expt. 2) and preserved at 4 °C.
In experiment 1 MFI and sarcomere length (SL) was measured on day 4 p.m. and SL and SF on day 8 p.m. on both muscles. Total and Soluble Collagen was determined on samples frozen on day 8 p.m. and preserved for about 1 1/2 month (-20 °C) on both LD and ST samples.
In experiment 2 MFI, SL and SF was determined on LD and ST (part A) samples frozen on day 8 p.m. and preserved by -20 °C for about 1 1/2 months and on ST (part B) preserved for 5 months p.m..

Methods

MFI was determined following the method of Culler et al (1978) with slight modifications (e.g. use of Ultra Turrax instead of Waring-Blendor), as will be described elsewhere.
SL was measured with the laser diffraction technique on fixed samples as described by Vandendriessche et al., (1984).
SF was measured on cooked samples (1 h, 75 °C) following the method recommended by the E.E.C. (Boccard et al., 1981). Cores with a diameter of 1.27 cm were taken perpendicular to the direction of the fibre bundles and sheared with a Warner-Bratzler shear mounted on an Instron model 1140 (Instron Ltd., High Wycombe).
The heat solubility of intramucular collagen (soluble collagen) was determined following the method of Sørensen (1981) with hydroxyproline determination according to ISO.DIS 3496.2. Soluble collagen was calculated as % of the sum of soluble and insoluble collagen (= Total collagen).

Results and discussion :

The standardization of the cooling conditions is illustrated by the mean temperatures on three times p.m. and the time necessary to reach 10 °C for both experiments and muscles (table 1). The cooling room temperature was somewhat higher in the second experiment (near 6 °C instead of 4 °C) and this has effected more the carcass temperatures of the LD than of the ST.

Table 1 : Mean temperatures (±SE) on three times p.m. and time necessairy to reach 10 °C.

	Temperature			Time to reach
	2.5h p.m.	4h p.m.	6h p.m.	10 °C
Long. Dorsi				
Expt.1 (n=26)	33.9 ± 0.3	27.3 ± 0.3	21.1 ± 0.3	14h40 ± 18 min
Expt.2 (n=34)	34.9 ± 0.3	30.0 ± 0.3	23.0 ± 0.3	16h42 ± 18 min
Semitendinosus				
Expt.1 (n=26)	31.5 ± 0.1	27.3 ± 0.1	23.5 ± 0.1	23h03 ± 14 min
Expt.2 (n=34 *)	32.1 ± 0.3	28.2 ± 0.2	24.2 ± 0.2	23h35 ± 22 min

* except for time to reach 10 °C n=32.

From table 1 it is also clear that the cooling conditions were not servere and were therefore not supposed to cause cold shortening. As is illustrated by the mean pH values in table 2, pH = 6.0 is reached within 2.5 to 4 hours in both mucles for most of the animals. This means that these young and well-nourished animals have a very fast post-mortem metabolism, limiting the risk of cold shortening. In table 2 the difference in p.m. metabolism of the two different mucles is clearly illustrated : the mean pH of the ST is in both experiments and on each measuring time significantly lower than the mean pH of the LD. For the ST the final pH is almost reached within 6 hours p.m. For both experiments the mean pH values for the same muscle on the same time p.m. are similar.
From table 1 and 2 it can be concluded that for all practical purposes standardization is reached.

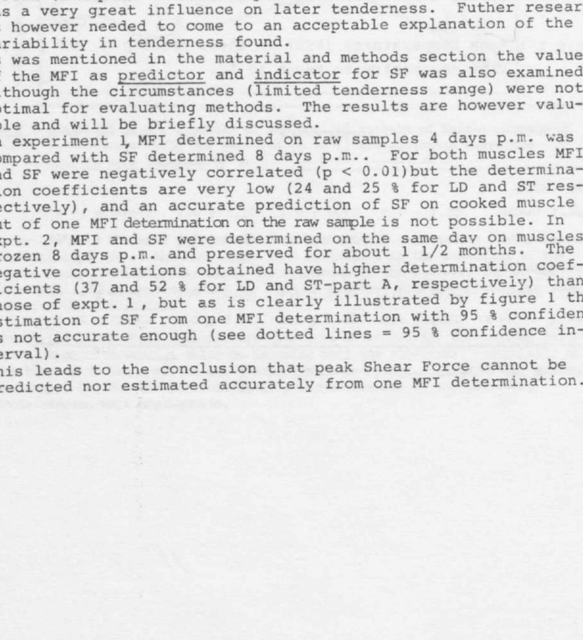
Table 2 : Mean pH and pH Difference/h

	pH				pH Diff./h in period		
	1 h	2.5 h	4 h	6 h	1-2.5	2.5-4	4-6
Expt.1 (n=26)							
Long. dorsi	6.90	6.30	5.95	5.71	0.398	0.235	0.122
Semitend.	6.73	6.03	5.67	5.48	0.466	0.243	0.095
Level sign.(a)	*	***	***	***	*	N.S.	N.S.
Expt.2 (n=33)(b)							
Long. dorsi	6.87	6.36	5.92	5.66	0.335	0.291	0.133
Semitend.	6.73	6.02	5.61	5.45	0.504	0.277	0.078
Level sign.(b)	*	***	***	***	***	N.S.	*

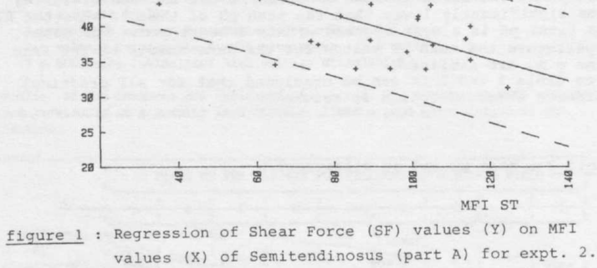
(a) Level of significance (t-test on means): * = $p < 0.05$; *** = $p < 0.001$; N.S. = Not significant.

(b) In experiment 2 one animal was omitted because of high end-pH (DFD-appearance): pH > 6.0 for LD and pH > 6.2 in ST. Because of this reason this animal is omitted from all further determinations.

In spite of this standardization there was still a considerable variability in peak Shear Force values for both experiments and muscles. For expt. 1 peak SF values (determined at 8 days p.m.) ranged from 25.1 to 81.9 N and from 33.9 to 57.6 N for LD and ST respectively. For expt. 2 these values (determined on samples frozen at 8 days p.m. and preserved for 1 1/2 months at -20 °C) ranged from 22.7 to 59.6 and 31.3 to 55.9 N for LD and ST respectivley (part A), and from 32.2 to 54.1 N for ST (part B) (preserved up to 5 months). This variability could not be related to breed differences (Red or Red-White). Some possible reasons for this variability in tenderness are discussed below.
As was first mentioned by Joseph & Connoly (1977) and later more thoroughly discussed by Lochner et al.(1980) and Marsh et al. (1981) the chilling conditions in the very early post mortem period may be crucial for tenderness enhancement after ageing. In both experiments there still existed slight differences in temperature. Maybe these slight differences have a great impact on tenderness, although there were no significant correlations between temperature on 2.5, 4 and 6 h p.m. and SF for both muscles and both experiments. A support for this possibility may be the fact that with the slightly higher cooling room temperatures of expt. 2 the tenderness range for the LD is smaller than for expt. 1, but there is no evidence that there is a causal effect. As the carcass temperature in our experiments is measured only from about 1 1/4 h p.m. on, we cannot take into account the influence of the temparature in the very first period p.m..
Another possible reason may be that the slaughtering procedure itself (the period immediatly before and during slaughtering) has a very great influence on later tenderness. Futher research is however needed to come to an acceptable explanation of the variability in tenderness found.
As was mentioned in the material and methods section the value of the MFI as <u>predictor</u> and <u>indicator</u> for SF was also examined, although the <u>circumstances (limited tenderness range)</u> were not optimal for evaluating methods. The results are however valuable and will be briefly discussed.
In experiment 1, MFI determined on raw samples 4 days p.m. was compared with SF determined 8 days p.m.. For both muscles MFI and SF were negatively correlated ($p < 0.01$) but the determination coefficients are very low (24 and 25 % for LD and ST respectively), and an accurate prediction of SF on cooked muscle out of one MFI determination on the raw sample is not possible. In expt. 2, MFI and SF were determined on the same day on muscles frozen 8 days p.m. and preserved for about 1 1/2 months. The negative correlations obtained have higher determination coefficients (37 and 52 % for LD and ST-part A, respectively) than those of expt. 1, but as is clearly illustrated by figure 1 the estimation of SF from one MFI determination with 95 % confidence is not accurate enough (see dotted lines = 95 % confidence interval).
This leads to the conclusion that peak Shear Force cannot be predicted nor estimated accurately from one MFI determination.



<u>figure 1</u> : Regression of Shear Force (SF) values (Y) on MFI values (X) of Semitendinosus (part A) for expt. 2. (n = 33). Regression equation: Y = 54.1 - 0.16 X.



<u>figure 2</u> : Regression of Shear force values (Y) of Semitendinosus on Shear force values of Long. dorsi (X) for expt. 1. (n = 26)
Regression equation: Y = 28.7 + 0.33 X

For experiment 1 Shear Force of both muscles (LD & ST) is very significantly correlated (r=0.6915, figure 2), but again the estimation of SF of ST from SF of LD is not accurate enough and this is in accordance with the conclusions of Dransfield & Jones (1981) who investigated the relationship between tenderness of three muscles. For expt. 2 the correlation between SF of the two muscles is smaller (r=0.3625), probably because the range of the SF values of the LD is smaller than in expt. 1.
The results of soluble and total collagen determinations and sarcomere length measurements did not help to explain tenderness variability, and will not be discussed here.
The conclusion of this work is that there is still a great variability in tenderness possible in muscles from bulls at standardized weight, age, slaughter and cooling conditions. As this tenderness variability cannot be explained from the variables measured, further research will be needed.

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