

## Tenderness of beef *m. longissimus lumborum* at normal and intermediate ultimate pH before and after a period of ageing

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**Abstract** Beef tenderness, in terms of Warner-Bratzler shear force values, was measured following ageing periods of 1 or 20 days for samples of *m. longissimus lumborum* from pairs of steers or heifers ( $n = 38$ ) with the two members of each pair being from the same mob. One sample of each pair had an intermediate ultimate pH (pHult; 5.7–6.2) and the other a normal pHult (<5.6). Samples of the intermediate pHult group had the expected lower cooking losses and darker colour (lower  $L^*$ ,  $a^*$ , and  $b^*$  values). In contrast to previous studies where intermediate pHult has been associated with tougher beef, however, the two groups did not differ in shear-force values, sarcomere length, or in myofibrillar fragility as measured by a filtration method or by image analysis. Samples for this study were removed from carcasses after *rigor mortis* had set in, which may have accounted for the sarcomere lengths being longer than in previous studies. It is suggested that the failure to detect a pHult effect on shear force values may have been because skeletal attachments prevented any pHult effect on sarcomere length. If this was the case, the problem of intermediate pHult toughness may be over-emphasised when muscle samples are removed from the carcass in the *prerigor* state. No evidence was found of a lower fragility of myofibrils at intermediate pH values.

**Keywords** beef; tenderness; ultimate pH; ageing

## INTRODUCTION

The relationship between ultimate pH (pHult) and toughness of beef has sometimes been reported to be curvilinear, with maximum levels of toughness at intermediate pHult levels (c. 5.8–6.2) (Bouton et al. 1957; Purchas 1990; Jeremiah et al. 1991; Devine et al. 1993; Purchas & Aungsupakorn 1993; Watanabe et al. 1996). Fjelkner-Modig & Ruderus (1983) also noted that maximum toughness occurred for beef with a medium pH between 5.81 and 6.19. Other studies, however, have shown a linear decrease in toughness over the whole pHult range from about 5.5 to 7.0 (Bouton et al. 1973a; Silva et al. 1999).

Bouton et al. (1973a) reported that the increased toughness with an increased pHult between 5.5 and 6.0 was not apparent in stretched beef deep pectoral muscle samples with a mean sarcomere length of 3.00  $\mu\text{m}$ , or in semitendinosus muscles with a mean sarcomere length of 2.30  $\mu\text{m}$ . Both these means are well above c. 1.90  $\mu\text{m}$ , below which decreases in sarcomere length are associated with increased Warner-Bratzler shear values (Bouton et al. 1973b). These results suggest that the toughening at intermediate pHult values is to some extent associated with fibre contraction state, and they are supported by reports that sarcomere lengths are shorter for intermediate pHult beef (Purchas & Aungsupakorn 1993; Olsson et al. 1995; Steen et al. 1997), and results showing that the lower toughness of normal-pHult beef is not apparent for samples that have cold-shortened (Bouton et al. 1973a; Purchas et al. 1988), probably because any pHult effect on sarcomere length was over-ridden by the low-temperature effect (cold-shortening). The failure to detect a curvilinear pattern by Silva et al. (1999) may also have been due in part to cold-shortening as the relatively light carcasses (100–164 kg) were chilled at 0°C without electrical stimulation, although sarcomere lengths were not particularly short.

It was suggested by Yu & Lee (1986) that the greater toughness at intermediate pHult may be due to lower proteolytic activity than at higher or lower pHult values, but if this is so, then it might be ex-

pected that intermediate-pH<sub>Hult</sub> meat would show less tenderness improvement with ageing. This was not the case with lamb *m. longissimus* in the study of Watanabe et al. (1996) where the toughness peak became flatter with ageing. Purchas et al. (1999) also reported that ageing effects were achieved at all pH<sub>Hult</sub> levels, with the largest absolute reduction in shear values at intermediate pH<sub>Hult</sub>, but in that study there were few samples in the intermediate pH<sub>Hult</sub> range. Simmons et al. (2000) found that intermediate pH<sub>Hult</sub> samples from several muscles improved with ageing, but normal pH<sub>Hult</sub> samples were not included in that study.

In the study reported here, samples of beef *m. longissimus lumborum* with intermediate pH<sub>Hult</sub> were collected from carcasses of commercial prime steers and heifers in order to compare the effects of a period of ageing on shear values and the myofibrillar fragility of beef of either normal or intermediate pH<sub>Hult</sub>.

## EXPERIMENTAL

### Meat samples

Samples of the full cross section of *m. longissimus lumborum* were obtained from the first to third lumbar vertebrae region of prime steers or heifers as 19 pairs, with one member of each pair being judged to be of intermediate pH<sub>Hult</sub> (pH<sub>Hult</sub> > 5.7 and < 6.2) based on a probe pH measurement on the morning following slaughter, and the second member being from a carcass from the same mob of cattle, but with a normal pH<sub>Hult</sub> (<5.6). No background information on the mobs of cattle or the individual carcasses that

the samples came from was collected, including whether the carcass was from a steer or heifer. Because the probe pH measurements may not have been true ultimate pH values, the values reported in this paper are those measured in an homogenate prepared after ageing (see below).

Samples were divided into two subsamples for ageing at c. 24 h *post mortem* with the 1-day samples being frozen (-20°C) immediately and the aged samples being stored at 3–3.5°C for 20 days before being frozen.

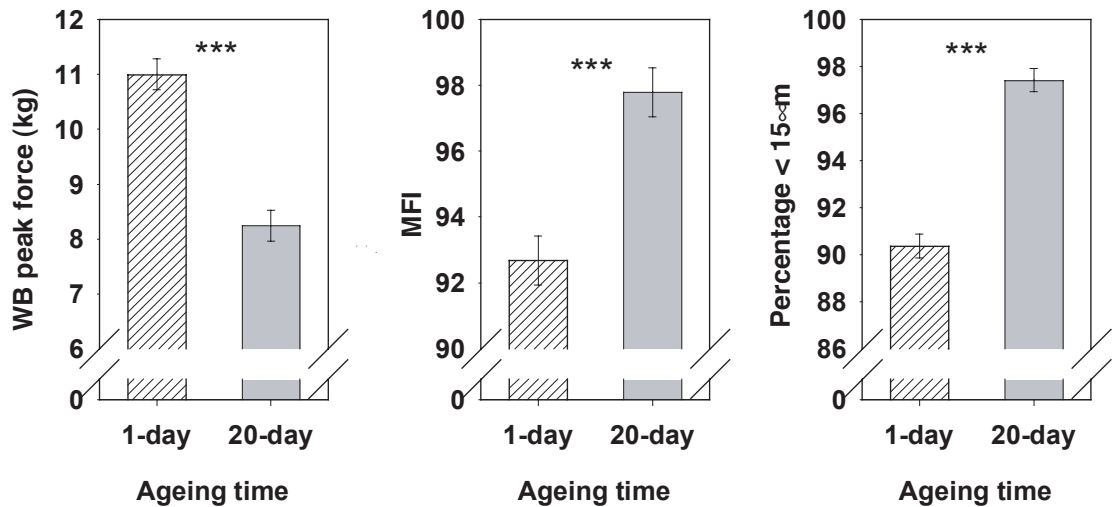
### Measurements

Measures of characteristics associated with tenderness were made using the procedures outlined by Purchas et al. (1997). Briefly, they included pH<sub>Hult</sub> (1.5–2.0 g muscle homogenised in 10 ml of neutralised 5 mM sodium iodoacetate containing 150 mM KCl), sarcomere length by laser diffraction, meat colour by reflectance spectroscopy using a Minolta Chromameter (L\*, a\*, and b\* values), Warner-Bratzler shear measurements using a square blade as described by Purchas & Aungsupakorn (1993) (after cooking at 70°C for 90 min and then cooling), and a myofibrillar fragmentation index (MFI) by a modification of the filtration method of Johnson et al. (1990) using a 231-µm-mesh stainless steel screen. The homogenising solution for MFI assessment differed from that of Purchas et al. (1997) in containing 0.05M EDTA as well as 0.85% NaCl.

In addition to being assessed by the filtration method, myofibrillar fragmentation was also measured by image analysis as follows. One millilitre of the homogenate prepared for filtration was added to 4 ml of homogenising solution and drops of this were used to prepare several microscope slides. Images

**Table 1** Means for cooking loss and image analysis parameters for samples of beef *m. longissimus lumborum* aged for either 1 or 20 days at 3–3.5°C. Ageing effects on other variables are shown in Fig. 1, and measures of the goodness-of-fit of the statistical model for the variables are shown in Table 3. *P* values, \*\*\* = *P* < 0.001; \*\* = *P* < 0.01.

	Ageing time (days)		
	1	20	Effect
Cooking loss (%)	24.0	24.3	0.14
Image analysis parameters:			
Av. fragment length (µm)	8.66	7.14	<0.0001***
Fragments < 5 µm (%)	20.5	24.4	0.003**
Fragments < 10 µm (%)	73.9	86.1	<0.0001***
Fragments < 20 µm (%)	96.0	99.4	<0.0001***



**Fig. 1** Differences between beef samples aged for 1 or 20 days for Warner-Bratzler (WB) peak force, myofibrillar fragmentation index (MFI), and percentage of myofibrillar fragments shorter than 15  $\mu\text{m}$  (means  $\pm$  SE). \*\*\* =  $P < 0.001$ .

were captured as jpg files using a video camera attached to a microscope at  $\times 400$  magnification. The images were analysed using an image analysis program (Bailey & Hodgson 1988), which scanned an area of about  $160 \times 160 \mu\text{m}$  and measured the length of items present that were accepted as myofibrillar fragments. Items were rejected as myofibrillar fragments for several possible reasons. These included (1) if the length was less than three times the width, (2) if the fragment was too bent, (3) if the edge was too irregular so that the perimeter was more than 50% greater than twice the sum of the length and width, and (4) if there was insufficient contrast with the background.

Several images were scanned with the aim of obtaining measurements on at least 200 fragments (average for this study = 331). The data were analysed by calculating the average fragment length and also the percentage of fragments with lengths of less than 5, 10, 15 or 20  $\mu\text{m}$ .

### Statistical analysis

Data analysis using PROC GLM within SAS (1985) was by a randomised block design with the two ageing times being nested within animals. Thus, each pair of carcasses was treated as a block and the pH-level effect and pair (block) effect were tested against the pair by pH-level interaction. The ageing-time effect and the pH-effect by ageing-time interaction were tested against the overall error.

## RESULTS AND DISCUSSION

### Ageing effects and measures of myofibrillar fragmentation

The changes with ageing (Fig. 1, Table 1) showed the expected pattern with a decrease in Warner-Bratzler (WB) shear parameters, an increase in MFI scores, a decrease in the average length of myofibrillar fragments, and an increase in the percentage of myofibrils with lengths less than 5, 10, 15, and 20  $\mu\text{m}$ . Cooking loss was unaffected by ageing.

Interrelationships amongst some of the characteristics related to myofibrillar fragility, and between them and either pH<sub>Hult</sub> or Warner-Bratzler peak force (WB-PF), are shown in Table 2, with correlations shown within samples aged for 1 and 20 days. These indicate that although relationships between MFI and measures of myofibrillar fragment length by image analysis were in the expected directions, and were significant in most cases, they were only moderately close for the overall dataset (data not shown), and were mainly low within an ageing time. There were highly significant negative correlations between average fragment length and the percentage less than 5, 10, 15 or 20  $\mu\text{m}$ , and highly significant positive correlations between these three percentages (Table 2). These results suggest that no one of these parameters obtained by image analysis stood out as being markedly superior to the others, but that the percent-

age of fragments less than 15  $\mu\text{m}$  or less than 20  $\mu\text{m}$  was marginally more useful than the others.

Correlations between myofibrillar fragment size and pHult or WB-PF (Table 2) were generally in the expected directions so that having more short fragments was associated with lower WB-PF values and, to a lesser extent, with a higher pHult, but the relationships were not very close. Neither were relationships clearly different between samples aged for 1 or 20 days, with the exception that the tendency for lower WB-PF values to be associated with higher MFI values was more apparent for the 1-day samples than the 20-day samples. This contrasts with the results of Purchas et al. (1999) where a stronger relationship was shown for 20-day samples, but the range of pHult values was much wider in that study. Generally, the correlations were higher when data for both ageing times were combined (data not shown), presumably because this resulted in a wider variation for most of the variables.

The high negative correlations between average fragment length and the percentage of fragments with lengths less than 5, 10, 15 or 20  $\mu\text{m}$  were as expected, as were the positive correlations between these three percentages (Table 2).

### Effects of ultimate pH

Means for the normal and intermediate pHult groups shown in Table 3 indicate that there was a clear difference in pHult as planned, but that this was not reflected in differences in Warner-Bratzler shear

values. The interaction between pHult-group and ageing-time tended to be significant for Warner-Bratzler peak force ( $P = 0.072$ ), reflecting the fact that the pHult effect was not significant at 1 day of ageing (10.95 versus 11.05 kg for normal and intermediate pH groups, respectively), but after 20 days the values were significantly lower (8.92 versus 7.56 kg for normal and intermediate pH groups, respectively;  $P < 0.05$ ) for the intermediate-pHult group. This result is opposite to the trend shown in several other studies for this pHult range (e.g., Purchas et al. 1999) where samples with an intermediate pHult have had higher shear-force values.

There was a tendency for sarcomeres to be shorter for the intermediate-pHult group ( $P = 0.06$ ), but the sarcomere lengths for both groups were appreciably longer than those reported by Purchas et al. (1999) and for other earlier studies from this laboratory that used muscle samples removed from the carcass within 90 min *post mortem*. Several of the other studies outlined in the Introduction that have shown an increased toughness with increasing pHult from 5.5 to about 6.0 have used muscle samples removed from carcasses in the *prerigor* state. In the current study muscles remained on the carcass until the day following slaughter, which may have been responsible for them entering *rigor mortis* in a more stretched state, thereby decreasing any effect pHult might have had on sarcomere length had the samples been free to shorten. If this was the case, then it suggests that the toughening associated with an in-

**Table 2** Linear correlation coefficients between pHult, Warner-Bratzler peak force (WB-PF), myofibrillar fragmentation index (MFI), and measures of myofibrillar fragment size for samples aged for 1 or 20 days ( $n = 38$ ).  $P$  values, \*\*\* =  $P < 0.001$ ; \*\* =  $P < 0.01$ ; \* =  $P < 0.05$ ; + =  $P < 0.10$ .

	Av. $\mu\text{m}$	% < 5 $\mu\text{m}$	% < 10 $\mu\text{m}$	% < 15 $\mu\text{m}$	% < 20 $\mu\text{m}$	MFI
At 1 day of ageing ( $n = 38$ ):						
pHult	-0.28+	0.06	0.29+	0.32+	0.23+	-0.05
WB-PF (kg)	0.39*	-0.07	-0.27+	-0.46**	-0.51**	-0.64**
% < 5 $\mu\text{m}$	-0.62***	-	-	-	-	-
% < 10 $\mu\text{m}$	-0.94***	0.64**	-	-	-	-
% < 15 $\mu\text{m}$	-0.94***	0.46**	0.88***	-	-	-
% < 20 $\mu\text{m}$	-0.83***	0.24	0.67***	0.86***	-	-
MFI	-0.39*	0.03	0.25	0.41*	0.52***	-
At 20 days of ageing ( $n = 38$ ):						
pHult	-0.19	0.10	0.09	0.32*	0.28+	0.14
WB-PF (kg)	0.42**	0.31+	-0.37*	-0.36*	-0.42**	-0.07
% < 5 $\mu\text{m}$	-0.73***	-	-	-	-	-
% < 10 $\mu\text{m}$	-0.94***	0.58***	-	-	-	-
% < 15 $\mu\text{m}$	-0.82***	0.33*	0.76***	-	-	-
% < 20 $\mu\text{m}$	-0.78***	0.30+	0.69***	0.90***	-	-
MFI	-0.18	-0.08	0.12	0.37*	0.33	-

crease in pH<sub>ult</sub> from 5.5 to 6.2 may be less of a problem in many normally hung carcasses, and may go some way towards explaining the variable results obtained in the different studies reviewed in the Introduction.

Other differences between the two pH<sub>ult</sub> groups shown in Table 3 are generally in the expected directions, with the intermediate-pH group having lower cooking losses and lower values for L\*, a\*, and b\* ( $P < 0.001$ ). Differences between the pH<sub>ult</sub> groups in MFI values and the percentage of fragments less than certain lengths were small and non-significant ( $P > 0.10$ ), except for average fragment

length, which tended to be shorter for the intermediate pH<sub>ult</sub> group ( $P < 0.10$ ); a difference that was consistent with the difference in Warner-Bratzler peak force values after 20 days.

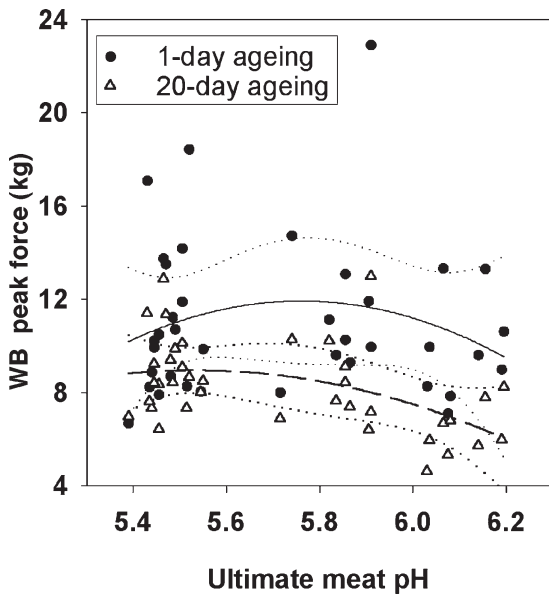
Table 4 shows that the extent to which several characteristics changed over the extra 19 days of ageing was similar for most variables for samples in the two pH<sub>ult</sub> groups. The exceptions were for Warner-Bratzler peak force where there was a greater reduction for the intermediate-pH group ( $P < 0.05$ ), and for cooking loss (%) ( $P < 0.0001$ ). However, there is no apparent explanation of why cooking loss should have increased with ageing for

**Table 3** Means for Warner-Bratzler (WB) shear values and other characteristics related to tenderness for samples of beef *m. longissimus lumborum* with either normal or intermediate ultimate pH. MFI = myofibrillar fragmentation index.  $P$  values, \*\*\* =  $P < 0.001$ ; \*\* =  $P < 0.01$ ; \* =  $P < 0.05$ ; + =  $P < 0.10$ .

	Ultimate pH group			Pair effect	$R^2\%$ (RSD)
	Normal	Intermediate	Effect		
Ultimate pH	5.48	5.97	<0.0001***	0.73	91(0.12)
Sarcomere length (µm)	1.98	1.86	0.06+	0.13	66(0.19)
Cooking loss (%)	26.1	22.1	<0.0001***	0.02*	94(0.99)
MFI	95.4	95.1	0.78	0.03*	62(4.6)
WB peak shear force	9.93	9.30	0.31	0.03*	85(1.71)
Parameters from image analysis:					
Av. fragment length (µm)	8.06	7.74	0.06+	0.03*	85(0.61)
Fragments < 5 µm (%)	22.3	22.6	0.78	0.007**	65(5.3)
Fragments < 10 µm (%)	79.0	81.0	0.11	0.04*	86(4.7)
Fragments < 15 µm (%)	92.9	94.9	0.01*	0.09+	82(3.1)
Fragments < 20 µm (%)	97.2	98.2	0.08+	0.53	73(2.1)
Colour parameters:					
L* (lightness)	32.3	29.6	<0.0001***	0.10	80(1.4)
a* (redness)	16.6	13.4	<0.0001***	0.16	82(1.4)
b* (yellowness)	6.15	4.00	<0.0001***	0.57	83(0.79)

**Table 4** Mean changes with ageing (day-1 value minus day-20 value) for Warner-Bratzler (WB) shear values and other characteristics related to tenderness for samples of beef *m. longissimus lumborum* with either normal or intermediate ultimate pH. MFI = myofibrillar fragmentation index.  $P$  values, \*\*\* =  $P < 0.001$ ; \* =  $P < 0.05$ .

	Ultimate pH group			Pair effect	$R^2\%$ (RSD)
	Normal	Intermediate	Effect		
Change in WB peak force (kg)	2.03	3.49	0.03*	0.03*	74(1.84)
Change in MFI	-4.74	-5.50	0.65	0.03*	71(5.00)
Change in av. fragment length (µm)	1.64	1.40	0.42	0.69	45(0.92)
Change in % fragments < 10 µm	-13.6	-10.8	0.22	0.66	48(6.9)
Change in % fragments < 15 µm	-7.63	-6.50	0.48	0.79	41(4.8)
Change in cooking loss (%)	-1.42	0.74	<0.0001***	0.49	70(1.39)



**Fig. 2** Relationships between ultimate meat pH and Warner-Bratzler (WB) shear force for beef samples aged for 1 or 20 days. Quadratic regression lines with 99% confidence intervals are shown, with the  $R^2$  values being 0.034 and 0.230 for the 1-day and 20-day samples, respectively.

intermediate pH<sub>Hult</sub> samples, but decreased for normal pH<sub>Hult</sub> samples.

The absence of any suggestion that pH<sub>Hult</sub> affected the extent to which measures of myofibrillar fragment size were reduced by ageing offers no support for the suggestion by Yu & Lee (1986) that proteolytic activity may be lower at intermediate pH<sub>Hult</sub> values.

The relationship between Warner-Bratzler shear force and pH<sub>Hult</sub> was also assessed by regression analysis following 1 and 20 days of ageing as shown in Fig. 2. The results were consistent with those in Tables 3 and 4 in showing that for this set of data there was no clear change in shear force as pH<sub>Hult</sub> increased over the range from 5.4 to 6.2 for samples aged for either 1 or 20 days.

A factor that could possibly have contributed to the absence of a clear relationship between shear force and pH<sub>Hult</sub> in this study is the unknown background of the cattle that the beef samples came from. This effect was minimised by taking samples from prime steers and heifers only, by ensuring that pairs

of samples comprising one intermediate and one normal pH<sub>Hult</sub> came from animals from the same mob, and by including pair number as a factor in the statistical model. The fact that the pair effect was significant ( $P < 0.05$ ) for a number of variables (Tables 3 and 4) justifies its inclusion as a factor in the model in this way.

## CONCLUSIONS

The increase in toughness of beef that has been reported under some circumstances to accompany an increase in pH<sub>Hult</sub> from 5.5 to 6.2 was not shown in the work reported here after ageing for either 1 or 20 days. It is suggested that the toughness associated with intermediate pH<sub>Hult</sub> values may be less apparent when samples of *m. longissimus lumborum* are not removed from the carcass *prerigor*.

Allowing this muscle to set in *rigor mortis* while attached to the carcass appears to prevent the shortening that may account, at least in part, for the greater toughness of intermediate pH<sub>Hult</sub> samples that has been reported in a number of other studies.

Effects of pH<sub>Hult</sub> on the extent of myofibrillar fragmentation up to a pH<sub>Hult</sub> of 6.2 were not detected in this study.

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