The contribution of pH towards endopeptidase activity and small heat shock protein distribution in beef and its role with achieved tenderness

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Keywords: heat shock proteins, beef tenderness, intermediate pH, calpain, cathepsin

Introduction

The contribution of cathepsins and calpains to the tenderising process has been investigated in much detail over the past two decades (Koohmaraie and Geesink, 2006; Sentandreu et al., 2002). However questions remain as to why the rate of meat tenderisation is so variable in red meat. One reason for this variability is ultimate pH (pH_u). High pH_u ($pH_u > 6.3$) throughout the ageing process will provide a good environment for calpain activity whereas normal meat ($pH_u < 5.7$) provides an optimum environment for cathepsin activity. Intermediate (Int) and high pH ($pH_u > 5.7$) beef is frequently observed in New Zealand due to a pasture-fed production system (low calorie diet) combined with pre-slaughter stress resulting in high and variable meat tenderness (Lowe et al., 2004). The role of small heat shock proteins (sHSP) in the conversion of muscle to meat has also proved an enigma to meat science (Aubry et al., 2006). Several proteome studies have identified sHSP like alpha β-crystallin (aβC) and HSP27 are upregulated in *postmortem* meat (Bouley et al., 2004; Hwang et al., 2005; Sayd et al., 2006). Others have speculated that HSP must have a role in controlling the processes of meat tenderness and quality (Herrera-Mendez et al., 2006; Ouali et al., 2006). This study aimed to characterise the behaviour of these molecular chaperones in *postmortem* beef muscle and determine if they contribute to beef tenderness.

Materials and Methods

The *M. longissimus dorsi* (LD) was removed from 22 month old bulls (n = 39) after carcass dressing, and placed in a water bath at 15°C until ~22 hours post-mortem, when pH_u was measured. Each LD was cut into 5 sub samples which were aged at 15ºC for 1, 2, 4, and 7 days *post rigor* when shear force was determined using a MIRINZ tenderometer. aβC and HSP20 concentrations were determined using monoclonal antibodies by quantitative ELISA at 3 h and 22 h *postmortem*, while the solubility of the two HSPs were determined by immunoblotting. The enzymatic activity of μ-calpain and cathepsin-L in the sarcoplasmic fraction was determined using fluorescent substrates (Calbiochem). Enzymatic kinetic assays were performed in buffers at a pH optimised for each enzyme, pH7.4 for μ-calpain and pH5.5 for cathepsin-L.

Results and Discussion

A contour plot (Figure 1) shows the relationship between pH_u, ageing time and tenderness, clearly demonstrating the effect of pH_u on tenderness. On this basis the LD samples were assembled into three groups called low pH ($pH_u < 5.7$; n = 23), Int pH ($5.7 < pH_u < 6.3$; n = 10) and high pH ($pH_u > 6.3$; n = 6).

The levels of aβC and HSP20 at 3 h and 22 h *postmortem* are shown in **Table 1**. At 3 h *postmortem*, the level of aβC was highest in the Int pH group, but differences observed between these groups were not statistically significant. At *rigor*, the levels of sHSP were segregated according to meat pH_u, low pH_u samples had the lowest levels of aβC and HSP20 whereas high pH_u samples contained the most sHSP.

At 3 h *postmortem*, when the muscle pH in all samples was high (pH_u 6.95-7.37), the amount of HSP in the total and the soluble extracts as observed by immunoblot was equivalent, indicating that much of the available HSP was in the soluble phase (Figure 2). At *rigor* however, all low pH_u and one Int pH_u muscle (pH_u 5.73) contained no or little soluble aβC. Despite this low pH_u samples contained equivalent amounts of aβC protein in the total extracts from the same time points, suggesting that aβC had not undergone degradation but had become insoluble at low pH.

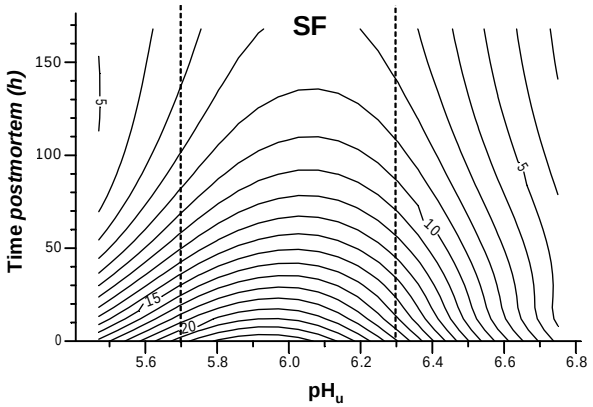

Figure 1 Contour plot showing the relationship between pH_u, ageing time and shear Kg-force (SF). The three pH groups are divided by vertical dashed lines.

Table 1 aβC and HSP20 concentrations expressed as a percentage of the total muscle protein for the three pH-groups at 3 and 22 h *postmortem* (pm)

		aβC		HSP20	
pH-group	n	3 h pm	22 h pm	3 h pm	22 h pm
Low	23	2.91	0.16	2.30	0.78
Intermediate	10	3.16	0.50	2.01	1.21
High	6	2.57	0.81	1.92	1.65
Max		6.37	1.33	4.82	2.76
Min		1.34	0.03	0.85	0.34

This pattern was replicated by HSP20, although the quantity transitioning between soluble and insoluble phases was less pronounced. The redistribution of sHSP at low pH was not replicated by the high molecular weight stress protein HSP70, which was consistently soluble in all samples (Figure 2).

The μ-calpain activity in the three pH groups was similar until *rigor*. High pH_u muscle maintained the highest level of μ-calpain activity for longest but displayed poor cathepsin-L activity after 48 h *post-mortem* (Figure 3). Low pH_u meat had the lowest levels of μ-calpain activity after 12h *postmortem* but displayed rising cathepsin-L activity after 48h. Int pH_u muscle exhibited intermediate μ-calpain and cathepsin-L activity throughout the *postmortem* ageing time. The measured μ-calpain and cathepsin-L activity was determined in buffers optimised for each enzyme system and not at the pH represented in each meat sample. Therefore the activity for both enzyme systems was suboptimal in Int pH_u muscles.

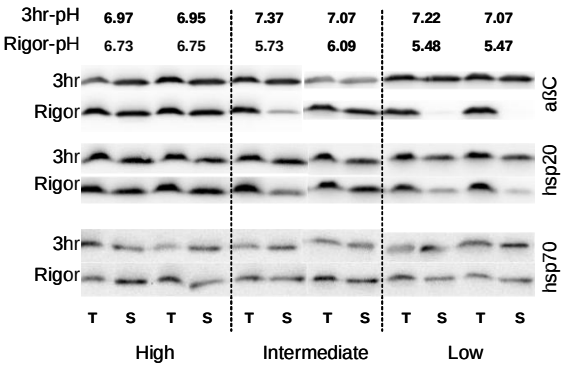

Figure 2 The solubility of HSP present in beef muscle at 3 h and 22 h *postmortem*. aβC, HSP20 and HSP70 were detected in equal volumes of total muscle extracts (T) or sarcoplasmic fractions (S).

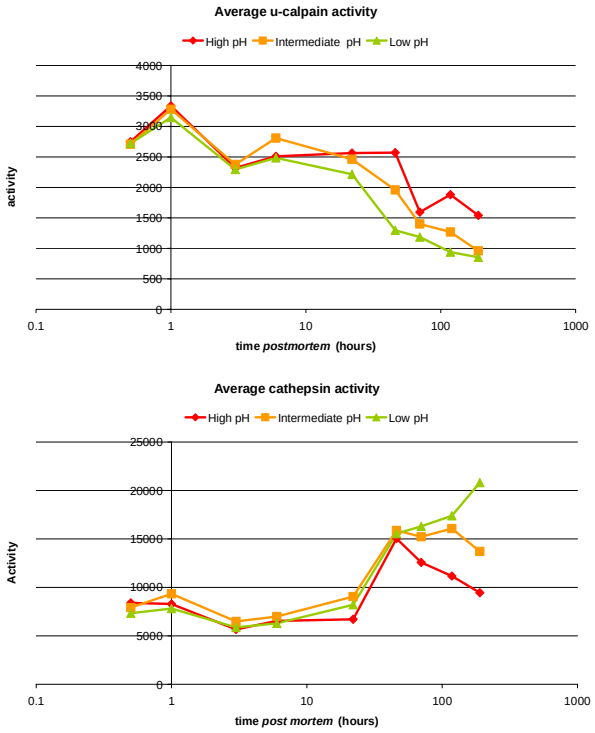

Figure 3 Average μ-calpain and cathepsin-L activity measured at ½, 1, 3 and 6 h as well as 1, 2, 4 and 7 days *postmortem* (time displayed on a log scale) for the high, intermediate and low pH_u groups.

Conclusion

Beef muscles with an intermediate pH_u displayed an extended time to achieve tenderness. This group exhibited higher levels of aβC protein expression at 3 h *postmortem* and contained a retained pool of soluble sHSP at the achieved pH_u. The combination of suboptimal cathepsin-L and μ-calpain activity and a soluble pool of sHSP protecting muscle structure provides a compelling explanation for why intermediate pH_u meat requires an extended ageing time.

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