Relation of postmortem protease activity to tenderness in buffalo meat and Brahman beef

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ABSTRACT: We previously showed that meat from crossbred water buffalo had significantly higher tenderness than beef from crossbred Brahman cattle of the same age, gender, and diet. Extensive studies on meat tenderness have indicated that proteases degrade muscle fibre proteins during postmortem storage, leading to weakening of the myofibrillar structure and an increase in tenderness. Thus, we investigated the difference in protease activity immediately postmortem, in order to explain the difference in tenderness between buffalo meat and beef. Five female crossbred water-buffalo (Philippine Carabao x Bulgarian Murrah) and five female crossbred cattle (Brahman x Philippine Native) were slaughtered at 30 months of age, and Longissimus thoracis muscle was sampled immediately post-slaughter. Protease activity at different pH levels and the effect of various inhibitors on protease activity were examined. Results showed that buffalo meat had significantly higher protease activity compared to beef, and calpain inhibitor 1 was the most effective inhibitor. As calpain inhibitor 1 is a specific inhibitor of calpain 1 and 2, the results suggest that higher calpain activity in buffalo meat was responsible for the higher tenderness of buffalo meat compared to Brahman beef.

Key words: Tenderness, Protease Activity, Inhibitor, Calpain.

INTRODUCTION - Meat from water buffalo (Bubalus bubalis) was recently shown to have significantly higher tenderness than beef from Brahman cattle of the same age, gender, and diet (Neath et al., 2007). There are extensive reports on the tenderisation process (for review see Koohmaraie and Geesink, 2006), indicating many variables that affect the tenderness of meat, in particular the postmortem degradation of key myofibrillar proteins by calpain enzymes during postmortem aging, which leads to weakening of the muscle structure and consequently an increase in tenderness. Therefore, the objective of the current study...
was to investigate the difference in protease activity, and the proteases responsible for this difference, between water buffalo meat and beef of the same age, gender, and diet.

**MATERIAL AND METHODS** - Five crossbred water-buffalo (Philippine Carabao x Bulgarian Murrah) and five crossbred cattle (Brahman x Philippine Native), all 2 year old females, were fed for 6 months on 50% fresh grass and 50% mixed concentrates at Philippine Carabao Center (PCC), and humanely slaughtered at Animal Products Development Center (APDC), Philippines. Muscle samples were collected from the caudal end of the Longissimus thoracis (LT) at 40 min postmortem, placed immediately in liquid nitrogen, and stored in a deep freezer at –80°C until analysis. Protease activity was determined by fluorescence spectroscopy using the substrate Suc-Leu-Leu-Val-Tyr-MCA (Peptide Inst., Inc., Japan). Total protease activity at pH 7.5, 6.7, or 6.4 was measured, followed by measurement of supernatant protease activity at pH 6.7 after purification by ultra-centrifugation, which separated the proteasome into the precipitate (Lamare *et al.*, 2002). A pH 7.5 is optimum for protease activity (Edmunds *et al.*, 1991), and pH 6.7 and 6.4 were the previously recorded pH levels for buffalo meat and beef respectively at 40 min postmortem (Neath *et al.*, 2007). Following a preliminary trial, the effect of calpain inhibitor 1 (specific inhibitor of calpain 1 and 2) on supernatant protease activity in buffalo meat and beef was further investigated on all ten buffalo meat and beef samples to confirm the findings.

**RESULTS AND CONCLUSIONS** - There was a tendency for higher protease activity in buffalo meat compared to beef at all pH levels as shown in Table 1, with a significantly higher protease activity of buffalo meat at pH 6.7 than beef. Furthermore, Table 1 shows supernatant protease activity was significantly higher in buffalo meat compared to beef. There was no significant difference between total and supernatant protease activity within buffalo meat or beef (Table 1), indicating minimal contribution of proteasome to the total protease activity of the two meats.

| Table 1. Total and supernatant protease activity (fluorescence signal/µg protein) at various pH levels, in beef and buffalo meat. (*P<.05). |
|---|---|---|---|
| **Total Protease Activity** | **Beef** | **Buffalo meat** | **Significance** |
| Ph 7.5 | 6.56 ± 0.33 | 7.55 ± 0.62 | NS |
| Ph 6.7 | 0.61 ± 0.04 | 0.92 ± 0.09 | * |
| Ph 6.4 | 0.38 ± 0.03 | 0.46 ± 0.05 | NS |
| **Supernatant Activity** | **Beef** | **Buffalo meat** | **Significance** |
| Ph 6.7 | 0.59 ± 0.14 | 7.97 ± 0.05 | * |

As there was a significant difference in protease activity between buffalo meat and beef, the effect of various inhibitors was measured to identify the major protease contributing to this difference in activity (results not shown). Of all the inhibitors tested, only calpain inhibitor 1 showed drastic inhibitory capability in both meats. Table 2 shows supernatant protease.
activity in buffalo meat and beef, with or without addition of calpain inhibitor 1, at pH 7.5 and 6.7. There was a clear reduction in protease activity due to the addition of calpain inhibitor 1 at pH 7.5, and 100% inhibition achieved at pH 6.7. In addition, buffalo meat had higher protease activity than beef, supporting the data presented in Table 1. These results confirmed that calpain inhibitor 1 caused significant inhibition of protease activity in both buffalo meat and beef, indicating major contribution of the calpain system to the difference between the two meats.

In this study, we showed that buffalo meat had significantly higher protease activity than beef. Furthermore, calpain inhibitor 1 was found to achieve significant inhibition of protease activity, suggesting that calpain 1 and/or calpain 2 were responsible for this difference in protease activity. These findings are consistent with previous reports that show a strong correlation between the calpain system and meat tenderisation postmortem (Koohmaraie & Geesink, 2006). In conclusion, higher calpain activity in early postmortem buffalo muscle is suggested to be responsible for the superior tenderness of water buffalo meat compared to Brahman beef.

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