## The effects of post-mortem storage and innovative processing technologies on cathepsin activities in meat

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**Introduction:** Endogenous enzymes have long been known to play an important role in meat tenderisation. Numerous studies have reported that µ-calpain could be important during initial aging while m-calpain could have a role in long term post-mortem aging (Kaur et al., 2021; Bhat et al., 2018). However tenderisation during aging has been proposed to be the result of synergistic action of many endogenous proteolytic systems and not only calpains (Cramer et al., 2018). Cathepsins, as they are found in the lysosomes, need rupturing of the lysosomes to be released into the cytosol for taking part in proteolysis. We hypothesised that cathepsins, primarily cathepsins B and L, if could be released into the cytosol through the use of innovative processing technologies, such as sous vide and pulsed electric field (PEF), will lead to enhancement of meat tenderness of not only tough beef meat cuts but also will shorten the post-mortem aging times. Also, the effects of post-mortem aging conditions were also determined on the activities of cathepsins in beef and goat meat.

**Materials and methods:** Post-mortem storage and sous vide treatment of beef: Hot-boned beef briskets (Deep and Superficial pectoral muscle) were used. The muscles were stored for either short-term (6 h to 4 d at either 4 °C or -20 °C) or long-term (further 4-18 d at either 4 °C or -20 °C) storage. Sous vide cooking of the meat was done in water baths set at different temperatures in the range of 50-70 °C for 1-24 h.

PEF treatment of beef: The treatment was carried out on beef briskets from Hereford sires heifers in a pilotscale batch PEF system (Elcrack-HVP 5, DIL, Quakenbruck, Germany). The electric field strength of 0.7 kV/cm to achieve a specific energy of 99  $\pm$  5 kJ/kg was used, at a constant pulse width and frequency of 20 µs and 50 Hz, respectively. Aging of goat meat: Goat muscle triceps brachii, from New Zealand female Boer cross (Boer XF) and female feral goats were stored after slaughtering at 4 °C for day 1, 4, 8 or 14 post-mortem; and their cathepsin activities and pH were determined.

Cathepsin assays: The activities of cathepsins B, B+L, H were determined based on the method from Chéret et al. (2007) with some modifications (Kaur et al., 2020) while the activity of cathepsin D was determined following the methods of Anson (1938) and Rico et al. (1991).

**Results:** Effects of post-mortemt storage conditions and sous vide cooking of beef: No significant changes in cathepsin H activity could be seen at any storage condition but an increase in cathepsin B activity was recorded during the initial storage (first 4 days) at both storage temperatures. Thereafter no change in their activities could be seen. Sous vide cooking of beef brisket at 50 °C during initial phases of heating led to a significant enhancement in cathepsins B+L activities. This increase could be correlated to meat tenderness usually seen in meat cooked at temperatures d 50 °C (Kaur et al., 2020).

PEF treatment of beef: Low intensity PEF did not show any significant differences in the specific activities of cathepsins in the cytosolic and lysosomal extracts of both control and PEF-treated raw samples.

Aging of goat meat: Breed significantly affected cathepsin activities, mainly cathepsins B and B + L. Boer XF had higher cathepsin B and cathepsin B + L activities than feral goats. Boer meat is more tender than feral so the results could indicate significant proteolytic degradation in the former as cathepsin B is known for its role in desmin degradation, impacting meat tenderness. For both breeds, there was no significant change in the activities of cathepsins B, B + L and H throughout the aging period while while cathepsin D increased until d 8 and showed a significant decrease at d 14. The pH of the meat from feral goats was in the range of intermediate pH (5.7-6.3), while the pH of Boer meat was high (pH > 6.3), which might be responsible for the observed differences in the enzyme activities among the two breeds.

**Conclusions:** Post-mortem storage conditions affected cathepsin activities, depending on the meat source. The findings also suggested that a multistage sous vide cooking with lower initial and higher final cooking temperature could be adopted to promote the enzymatic proteolysis to achieve optimum tenderness, while ensuring microbiological safety of meat. This is a work in progress.

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