# The Effect of pH on µ-calpain Activity and Implications in Meat Tenderness

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**Summary and Implications**

In early postmortem muscle, µ-calpain inactivation due to either a rapid pH decline or by rapid autolysis has the potential to decrease proteolysis of myofibrillar proteins and subsequent postmortem tenderization. An intermediate pH decline that allows for proteolytic activity of µ-calpain, but a slower rate of autolysis could explain a portion of the variation in meat tenderness.

**Introduction**

The significant changes in muscle intracellular environment that occur early postmortem are known to influence meat quality. One of the most pronounced changes is the pH decline from 7.5 in living muscle to approximately 5.6 in meat. µ-calpain, a $Ca^{2+}$-activated protease, is thought to be responsible for much of the postmortem proteolysis that occurs of myofibrillar and cytoskeletal proteins. The increase in tenderness observed in meat during postmortem storage is associated with proteolysis of these proteins. The objective of this study was to determine the effect of pH on µ-calpain activity and µ-calpain autolysis (self-proteolysis and inactivation).

**Materials and Methods**

µ-Calpain was purified from at-death porcine *semimembranosus* muscle. µ-Calpain proteolytic activity was determined using a fluorescence assay. The following pH and ionic strength conditions were used to determine the influence of pH and ionic strength on calpain activity: pH 7.5 and 165 mM NaCl; pH 6.5 and 165 mM NaCl; and pH 6.0 and 165 mM NaCl. Calpain activity was recorded at 30 and 60 min**.** Calpain autolysis in the same samples was determined using immunoblotting analysis.

**Results and Discussion**

µ-Calpain activity was greater at pH 6.5 compared to pH 7.5 and 6.0 (Table 1). In order to understand the mechanism underlying the greater activity of µ-calpain at pH 6.5, µ-calpain autolysis was examined using western blots. Autolysis, or self-degradation, is often used as an indicator of activation and inactivation of µ-calpain. The intact 80 kDa subunit of µ-calpain will autolyze to a 78 kDa subunit and further to a 76 kDa subunit. Early in the incubation, autolysis was fastest at pH 7.5 as indicated by disappearance of the intact 80 kDa band and the accumulation of the 76 kDa band, which most likely resulted in inactivation of µ-calpain. In contrast, at pH 6.5, autolysis occurred more slowly, thereby inactivation did not occur as quickly and likely contributed to the greater µ-calpain activity at 30 and 60 min. Autolysis observed at pH 6.0 shows greater intensity of the 78 kDa autolysis product and little accumulation of the 76 kDa band indicating less activation of µ-calpain.. Very little 78 kDa autolysis product was detected at pH 7.5 or 6.5 at all time points. This contrast indicates that pH affects the accumulation of the 78 kDa autolysis product.

Moderate rates of postmortem pH decline (pH of 5.8 to 6.2 at 3 h) have been shown to produce the most tender beef loin steaks, whereas rapid rates (pH of 5.5 at 3 h) and slow rates (pH 6.8 at 3 h) of postmortem glycolysis produced less tender meat. Greater µ-calpain activity at the intermediate pH used (pH 6.5) than pH of 7.5 and pH of 6.0 explains this effect of pH decline on meat tenderness. If the pH decline is rapid, µ-calpain activity is diminished due to the lower pH. If postmortem glycolysis is slow and the pH does not decline as rapidly, µ-calpain would autolyze earlier postmortem, thereby losing proteolytic activity earlier and not allowing for maximal proteolysis. Intermediate pH decline thus allows more proteolysis and slower completion of autolysis, therefore ultimately allowing for greater postmortem myofibrillar protein breakdown and increased tenderization.

**Table 1. Effect of pH on µ-calpain activity**

| | Activity[a] | |
|---|---|---|
| pH | 30 min | 60 min |
| pH 7.5 | 94.21[c] (2.50) | 152.53[c] (1.54) |
| pH 6.5 | 131.65[b] (6.79) | 208.18[b] (8.87) |
| pH 6.0 | 46.47[d] (3.20) | 68.12[d] (4.48) |

[a]Activity= fluorescence units with $CaCl_2$- fluorescence units with EDTA (n = 3).
[b,c,d] Within a column, means without a common superscript differ (*P<0.01*).
Value in parentheses = standard error of the mean