

ACTIVITIES OF THE CALPAIN SYSTEM ARE STABLE DURING FROZEN STORAGE OF MEAT AT -20°C AND -80°C

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Background

Meat samples used in research are often stored frozen for various time periods before e.g. determination of enzyme activity or sensory/instrumental determination of eating quality. Larger samples for Warner-Bratzler shear force determination are routinely stored at -20°C and smaller samples for determination of the calpain system at -80°C . Koohmaraie (1990) reports that the activities of μ -calpain and m-calpain are stable at -70°C for 6 weeks storage while the activity of calpastatin decreases during frozen storage. Only 45 % of the original activity of calpastatin could be measured after 6 weeks of frozen storage. The instability of calpastatin activity during frozen storage has also been observed by Whipple and Koohmaraie (1992) at -30°C for 4 weeks and by Duckett et al. (1998) at -20°C for 6 weeks. The mechanism underlying the reported instability of calpastatin in frozen meat is not understood.

Objectives

To study the stability of μ -calpain, m-calpain and calpastatin activity during prolonged freeze storage at -20°C and -80°C .

Methods

One porcine *longissimus dorsi* was obtained from a local slaughterhouse 45 minutes post-mortem (PM) and transported to the lab. Approximately 2 hours PM the muscle was cut into samples of 2 cm^3 . Using two random samples the activities of μ -calpain, m-calpain and calpastatin were determined (day 0). The remaining samples were frozen in liquid nitrogen. The frozen samples were mixed thoroughly and divided into two batches which were stored at either -20°C or -80°C for 20 weeks. Nine times during the storage period a sample was randomly selected from each batch for determination of extractable activity of μ -calpain, m-calpain and calpastatin. Activity measurements of enzymes and inhibitors were made according to Kristensen et al. (2002). Calpastatin assays require the use of partially purified m-calpain. The purification procedure consist essentially of two steps: separation of calpastatin from calpain using a hydrophobic interaction column and afterwards separation of μ -calpain and m-calpain using an ionexchange column. A stock solution of partially purified m-calpain was prepared the foregoing week and stored at 4°C in 50 % glycerol. Before measuring the activity of calpastatin in the samples, the activity of the m-calpain stock solution was determined and the solution was diluted to give an activity of 0.2 U/mL which was used for the calpastatin assay.

Results and Discussion

Figure 1 presents the activities of the calpain system during frozen storage at -20°C and -80°C . No difference seems to exist between the two storage temperatures for any of the three components of the calpain system. The extractable activity of μ -calpain and m-calpain did not change during the storage period indicating that the enzymes were stable at both temperatures. The activity of calpastatin was constant in the first few weeks of the storage period whereafter it decreased until day 123.

The decreased activity of calpastatin is in agreement with Koohmaraie (1990), Whipple and Koohmaraie (1992) and Duckett et al. (1998), however; these studies observed a more rapid decrease in activity than observed in **Figure 1**.

Meat samples are stored at -80°C because chemical and biological events are expected to be negligible at this temperature. Thus, it is surprising that calpastatin seems to be unstable at this temperature and that no differences is observed between storage at -20°C and -80°C . **Figure 2** shows that the activity of the m-calpain stock solution decreased during storage in 50 % glycerol at 4°C . We hypothesized that the observed decrease in calpastatin activity was related to the changes in m-calpain stock solution. At day 143 the activity of calpastatin was therefore assayed using a newly purified stock solution of m-calpain. The resulting calpastatin activity was similar with the initial levels observed at the start of the storage period (**Table 1**). The new stock solution gave a 44 % higher calpastatin activity at day 143 than the old stock solution and there was no difference between the calpastatin activity originally measured at day 0 and calpastatin activity measured at day 143 using the new stock solution. Thus, the observed decrease in calpastatin activity during frozen storage most probably is due to instability of the m-calpain stock solution. The m-calpain used in the calpastatin assay is adjusted to 0.2 U/mL. However, the dilution factor decreased because of the activity loss during the storage period. Therefore increased the concentration of the m-calpain molecules in the adjusted solution, used in the assay, with increasing storage time of the stock solution. If calpastatin binds to inactivated m-calpain in the assay and the inactive m-calpain molecules thereby mask the binding sites on calpastatin for active m-calpain molecules, then the calpastatin activity would appear to be less than it actually is. This suggestion could explain the observed decrease in calpastatin activity during frozen storage.

Conclusions

- The extractable activities of μ -calpain, m-calpain and calpastatin from meat are constant during frozen storage at -20°C and -80°C for at least 18 weeks. This imply that both the calpains and calpastatin are stable when stored frozen.
- The observed decrease in calpastatin activity is probably caused by instability of the m-calpain solution used in the calpastatin assay.
- The activity of m-calpain stored at 4°C in 50 % glycerol is decreased to 18 % of the initial value when stored for 18 weeks.

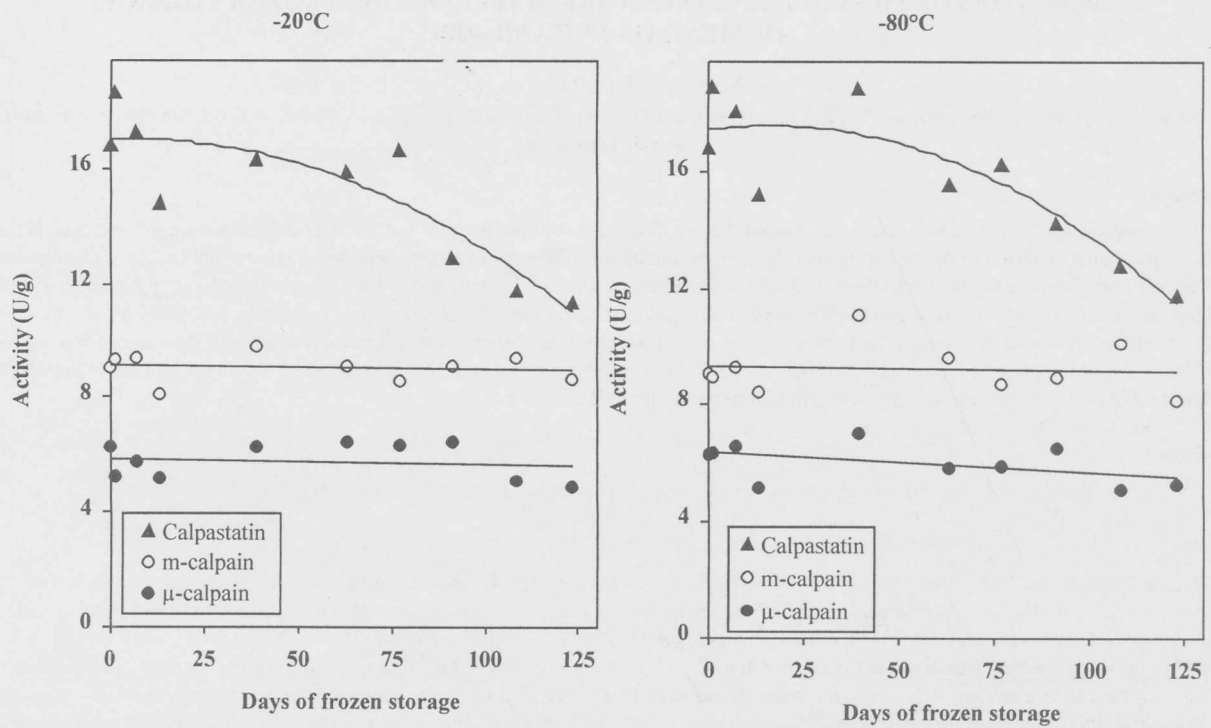


Figure 1. Activity of μ -calpain, m-calpain and calpastatin during storage at -20°C or -80°C for 18 weeks. Single determination at each point. The same stock solution of m-calpain was used for calpastatin determination until day 123.

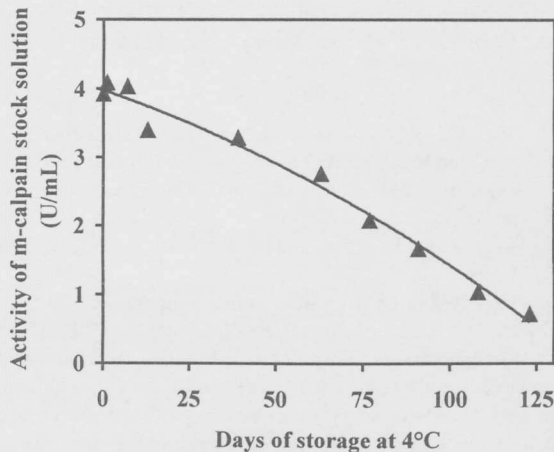


Figure 2. Activity of m-calpain stock solution.

Table 1. Calpastatin activity measured using old or new stock solution of m-calpain. Double determination on meat stored at -80°C .

	Old		New
	Day 0	Day 143	Day 143
U/g	16.8	11.5	16.6
sd	1.5	0.8	0.8

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