THE EFFECT OF TEMPERATURE AND TIME ON ACTIVITY OF CALPAINS AND LYSOSOMAL ENZYMES AND DEGRADATION OF DESMIN IN PORCINE LONGISSIMUS MUSCLE

Per Ertbjerg[1], Liv S. Christiansen[2], Anders B. Pedersen[2] and Lars Kristensen[3]

[1]Department of Food and Environmental Sciences, P.O. Box 66, FI-00014 University of Helsinki, Helsinki, Finland

[2]Department of Food Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

[3]Danish Meat Research Institute, Maglegårdsvej 2, DK-4000 Roskilde, Denmark

Abstract – The experiment was conducted to determine the effect of temperature and time during heat treament on the activity of µ- and m-calpain and cathepsin B + L. In addition the degradation of the structural protein desmin was studied. Porcine *longissimus* muscle was sampled at 24 hours postmortem and incubated at temperatures of 25, 40, 55 and 70°C for the next 24 hours. At 25°C µ-calpain lost most of its extractable activity within the first 3 hours, whereas m-calpain was more stable. At 40 °C m-calpain activity disappeared within 3 hours. At 55°C µ-and m-calpain was rapidly inactivated, whereas cathepsin B + L was remained active throughout the heat treatment up to 24 hours. At 70°C calpains were inactivated within 10 minutes, cathepsin B + L within 90 minutes and desmin was not degraded. Desmin was degraded more rapidly at 40°C than at 25°C, whereas less degradation occurred at 55 °C. In conclusion, the data suggests that meat proteolysis is accelerated during the initial stages of cooking. Calpains were active for 1 to 3 hours at 40°C. Cathepsin B and L remained active at 55°C, but only around 1 hour at 70°C.

Key Words – cooking, pork, proteolysis

I. INTRODUCTION

The calpain system has been shown to be an important contributor to proteolytic degradation of myofibrillar proteins during storage of meat. Especially µ-calpain is thought to be responsible for many of the proteolytic changes that occur during conditioning. The role of m-calpain in post-mortem tenderization has been questioned, as the calcium concentration in post-mortem muscle may not to be high enough for activation. In porcine muscle, however, not only µ-calpain, but later also m-calpain has potential to autolyse during post-mortem storage at refrigerated temperature [1]. The sarcoplasmic calcium level in porcine muscle rises under certain time-temperature combinations to the level required for activation of m-calpain, and increased temperatures of 15 to 30ºC have been shown to activate m-calpain [2]. Although the calpain system should be considered to be mainly responsible for texture development in meat, an increasing number of proteases, including the lysosomal cathepsins, have been implicated in making a contribution to postmortem proteolysis, thereby supporting the view that postmortem protein degradation is multi-enzymatic in nature. Both µ- and m-calpain as well as cathepsin B have been demonstrated to degrade desmin [3]. In addition to proteolysis during ageing, enzymatic activity during cooking may have an additional effect on the tenderness development, especially if the meat is kept for a longer period of time at optimal temperatures for enzyme activity [4]. In a study on holding temperatures the ageing rate increased exponentially up to 40°C and the temperature coefficient ($Q_{10°C}$ = 2.4) was typical of an enzymatic reaction [5]. Maximum ageing rate occurred near 60°C, was still high at 65°C, and then declined to a low level at 75°C. How proteolytic enzymes operate during cooking of meat and their relation to the tenderization at different temperatures is not well understood. The purpose of this study is to investigate the activity of µ- and m-calpains and cathepsines B + L along with the degradation of desmin during heat treatment of pig muscle at different holding temperatures (25, 40, 55 and 70°C).

II. MATERIALS AND METHODS

At 24 h post mortem *longissimus dorsi* from one conventional raised pig was removed, and were

cut into strips of 5 cm along the fibre (0.5 × 1 x 5 cm) and then vacuum packaged. The meat strips were cooked immediately in water bath at 25, 40, 55 and 70°C for 2, 10, 40, 90 min and 3, 5 and 24 h. The samples were then frozen at -20°C and stored until analysis of m-calpain, µ-calpain, cathepsin B+L activity and desmin level. The µ- and m-calpain activity was determined by casein zymography as described in [1], the cathepsin B and L activity was determined as described in [6] and desmin degradation was measured as described in [7].

III. RESULTS AND DISCUSSION

At all temperatures an initial large drop of µ-calpain occurred within 10 minutes of heat treatment (Fig. 1). At 25°C µ-calpain remained active during the heat treatment, but lost most of its extractable activity within 3 hours. At 40°C all extractable activity was lost within 3 hours. No extractable activity was measured after 10 min at 55°C or 2 min at 70°C.

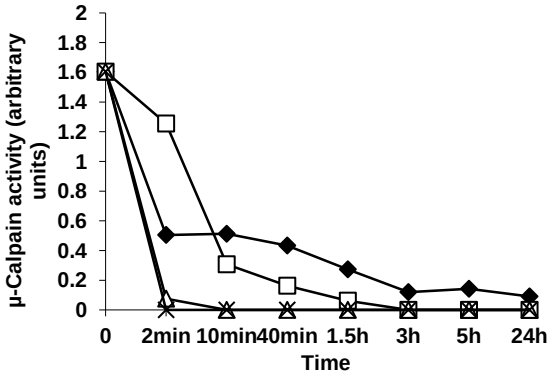

Fig. 1. Extractable µ-calpain activity from porcine *longissimus* muscle heat treated at ◆ 25; □ 40; △ 55; and × 70°C for 0, 2, 10, 40 min and 1.5, 3, 5, 24 hours.

Extractable m-calpain was more stable at 25 °C than at higher temperatures (Fig 2). No significant ($P > 0.05$) change in m-calpain activity was observed within 5 hours of heat treatment, but thereafter the activity declined ($P < 0.001$). At 40°C m-calpain decreased ($P < 0.001$) and from 3 hours and onwards no activity was measured. No

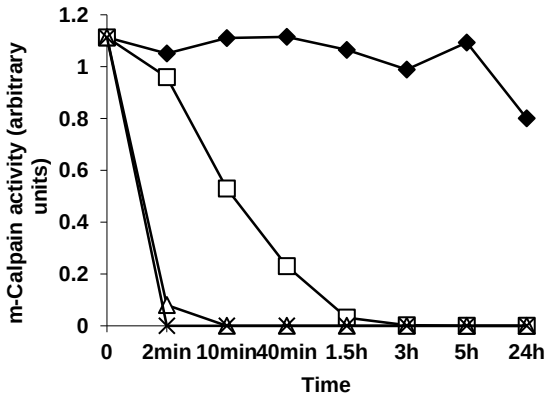

Fig. 2. Extractable m-calpain activity from porcine *longissimus* muscle heat treated at ◆ 25; □ 40; △ 55; and × 70°C for 0, 2, 10, 40 min and 1.5, 3, 5, 24 hours.

extractable activity was measured after 10 min at 55°C or 2 min at 70°C. Calpain activation and autolysis by calcium eventual lead to loss of proteolytic activity. This is probably due to thermodynamic instability of partially autolyzed calpain. However, in a rapid heating process the extractable activity may also be lost due to heat-induced denaturation of the native enzyme without prior activation and autolysis. Incubation at the lower temperature of 25°C resulted in formation of the autolyzed forms of both µ- and m-calpain (Fig. 3). The presence of the autolyzed forms indicates that both enzymes were active during the incubation period of 24 hours. µ-Calpain was activated earlier than m-calpain, but the active form of µ-calpain also disappeared more rapidly. After 24 hours only a small fraction of the original µ-calpain activity remained, whereas m-calpain was still being activated. These data shows that temperature activates m-calpain in a time-dependent manner, in agreement with recently published results [2].

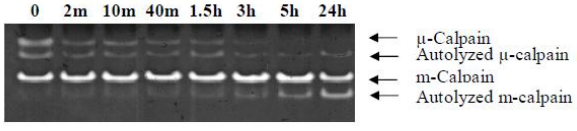

Fig. 3. Casein zymograms showing activity of native and autolyzed µ- and m-calpain from pig *longissimus* heat treated at 25°C for up to 24 hours.

Extractable cathepsin B and L activity was much more heat stable and remained active throughout heat treatment at 25, 40 and 55°C (Fig. 4).

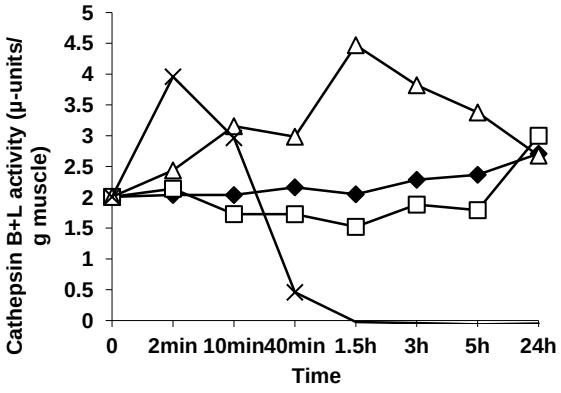

Fig. 4. Activity of extractable cathepsin B+L from pig *longissimus* muscle heat treated at ◆ 25; □ 40; △ 55; and × 70°C for 0, 2, 10, 40 min and 1.5, 3, 5, 24 hours.

At 70°C the activity increased after 2 min, but was lost after 1.5 hours due to heat-induced inactivation. At 55°C cathepsin B + L activity increased ($P < 0.01$) with cooking time and reached a maximum after 1.5 hours. These data suggests that part of cathepsin B + L in porcine *longissimus* exist in the form of a pro-enzyme, which become activated by heat.

Desmin was degraded at elevated temperatures of 25°C and more rapidly at 40°C (Fig. 5). At 40°C a substantial degradation of desmin occurred within 3 hours. After 24 hours heat treatment at 55°C only a minor amount of desmin was degraded and at 70°C desmin degradation was not clearly visible. The reduced or absent desmin degradation at 55 and 70°C suggests that the proteolytic enzyme system specific for desmin is thermal unstable at these temperatures. The lack of degradation of desmin in pig muscle heat treated at 70°C is in agreement with the observation that all the measured proteolytic enzyme systems were inactivated. However, at 55°C cathepsins B + L were fully active, whereas the calpains had lost activity within 2 to 10 min.

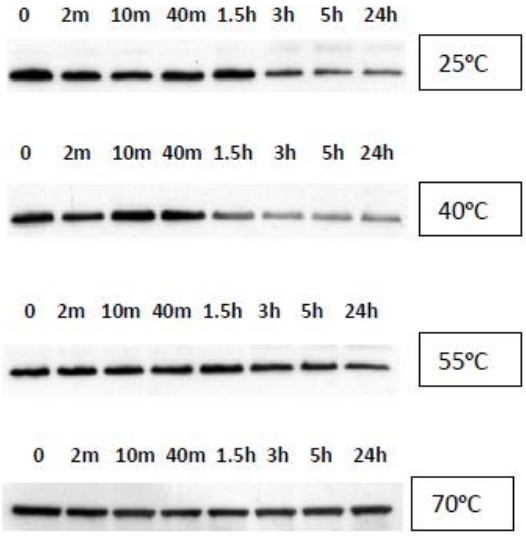

Fig. 5. Western blot against desmin on myofibrillar proteins extracted from pig l*ongisimus* muscle after heat treatment at 25°C, 40°C, 55°C and 70°C for 0, 2, 10, 40 min and 1.5, 3, 5, 24 hours.

If the cathepsins were the main enzymes responsible for desmin degradation, a high level of degradation would be expected at 55 °C. This was not observed. It can be argued, however, that the cathepsins had not yet had time to be released from the lysosomes in this experiment, as the heat treatment started 24 hours post mortem.

Although both calpains were rapidly inactivated, they may have had time enough to induce some desmin degradation at 55°C. Therefore the data points to that calpains are more involved in desmin degradation during the initial stages of cooking as compared to the cathepsins.

IV. CONCLUSION

In conclusion, the data suggests that meat proteolysis is accelerated during the initial stages of cooking. Calpains were active for 1 to 3 hours at 40°C. Cathepsin B and L remained active at 55°C, but only around 1 hour at 70°C. Overall, the data points to that calpains are more involved in desmin degradation during the initial stages of cooking as compared to the cathepsins.

ACKNOWLEDGEMENTS

The technical assistance on casein zymography of Julie Cecelia Stefansen from University of Copenhagen is gratefully acknowledged.

REFERENCES

1. Pomponio, L., Lametsch, R., Karlsson, A. H., Costa, L. N., Grossi, A. & Ertbjerg, P. (2008). Evidence for post-mortem m-calpain autolysis in porcine muscle. Meat Science 80: 761-764.
2. Pomponio, L. & Ertbjerg, P. (2012). The effect of temperature on the activity of μ- and m-calpain and calpastatin during post-mortem storage of porcine longissimus muscle. Meat Science 91: 50-55.
3. Baron, C. P., Jacobsen, S & Purslow, P. P. (2004) Cleavage of desmin by cysteine proteases: Calpains and cathepsin B. Meat Science 68: 447-456.
4. Christensen, L, Ertbjerg, P., Aaslyng, M.D., & Christensen, M. (2011) Effect of prolonged heat treatment from 48 degrees to 63 degrees on toughness, cooking loss and color of pork. Meat Science 88: 280-285.
5. Davey, C. L. & Gilbert, K. V. (1975). The temperature coefficient of beef ageing. Journal of Food Science and Agriculture 27: 244-250.
6. Ertbjerg, P., Larsen, L. M. & Moller, A. J. (1999) Effect of prerigor lactic acid treatment on lysosomal enzyme release in bovine muscle. Journal of the Science of Food and Agriculture 79: 95–100.
7. Kristensen, L. & Purslow P.P. (2001) The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. Meat Science 58: 17-23.