EFFECT OF PRE-RIGOR TEMPERATURE INCUBATION ON SARCOPLASMIC PROTEIN SOLUBILITY, CALPAIN ACTIVITY AND MEAT QUALITY IN PORCINE MUSCLE

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Abstract – The aim of the study was to investigate the effect of pre-rigor temperature incubation on enzyme characteristics in relation to meat quality within porcine muscle. Porcine *Longissimus dorsi* muscles were incubated at temperatures of 0, 10, 20, 30 and 40 ºC to 6 h post mortem. Incubation at 40 ºC induced a significant decrease of sarcoplasmic protein solubility and an increase in proteins in the myofibrillar fraction. SDS-PAGE and Western blot suggested that the enzymes phosphorylase and creatine kinase precipitated onto the myofilaments during incubation at 40 ºC. Higher drip loss was found following 40 ºC incubation. The precipitation of phosphorylase and creatine kinase could be a causative factor of reduced water-holding at the combination of high temperature and low pH. Incubation at 40 ºC resulted in substantially lower shear force in parallel with loss of extractable activity of µ- and m- calpain, suggesting a rapid activation of both enzymes in porcine at high temperatures and low pH early post mortem.

Key Words – Glycolysis rate, Early pH decline, Glycogen phosphorylase, Creatine kinase, Water-holding capacity, Meat tenderness

I. INTRODUCTION

It is generally agreed that PSE meat development is a result of abnormally fast glycolysis perimortem. The combination of high temperature and low pH is able to induce protein denaturation, which is a decisive factor also of reduced water-holding capacity of meat [1]. It is believed that the denaturation of the sarcoplasmic proteins is an important attribute to the poor water-holding capacity of PSE meat [2]. Phosphorylase and creatine kinase disappear from the sarcoplasmic fraction of PSE meat [3,4]. In addition, by high temperature incubation with low pH, it is possible to obtain similar denaturation of glycogen phosphorylase [5, 6] and creatine kinase [7] as in PSE meat. Different hypotheses have been proposed to explain the reduced water-holding capacity of PSE meat in relation to protein denaturation [2, 6, 8]. However, further studies to reveal the relationship between pre-rigor temperature and water-holding in relation to protein characteristics are needed. In order to investigate the influence of early post mortem temperature on the characteristics of sarcoplasmic proteins, the present study induced differences in glycolysis rate by pre-rigor incubation of porcine *Longissimus dorsi* muscles at 0, 10, 20, 30 and 40 ºC. The solubility of phosphorylase and creatine kinase as well as calpain activity and meat quality parameters were investigated.

II. MATERIALS AND METHODS

Porcine *Longissimus dorsi* muscles were divided for five temperature incubations from 50 min to 6 h post mortem. pH and temperature were recorded. Sarcomere lengths, drip loss and Allo-Kramer shear force were measured at several time points. Myofibrillar and sarcoplasmic fractions were separated by muscle homogenization in rigor buffer (75 mM KCl, 10 mM KH_2PO4, 2 mM $MgCl_2$, 2 mM EGTA, pH 7.0) and following centrifugation at 10,000 x g at 4 °C for 10 min each time [3]. Same amount of proteins from both fractions were loaded to 7% Tris-Acetate gels (Invitrogen) for SDS-PAGE. Proteins were then transferred to Immobilon-FL Transfer Membrane (Millipore, Bedford, MA) for Western blot of phosphorylase and creatine kinase. Calpain analysis was done by casein zymograhy [9].

III. RESULTS AND DISCUSSION

Differences in temperature during incubation were able to alter the pH decline rate (Fig.1). Muscles incubated at 40 °C had significantly faster pH decline, indicating that the combination of low

ultimate pH of 5.5 occurred simultaneously with high temperature only in these samples.

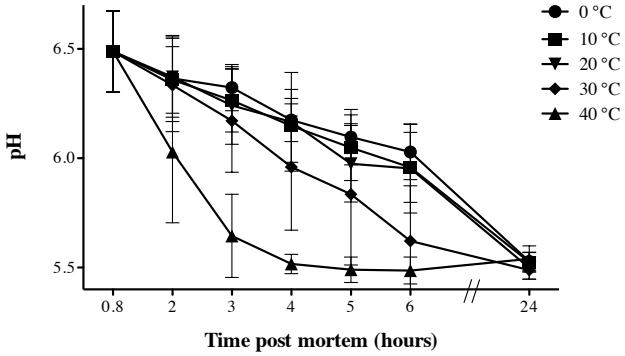

Figure 1. pH of *Longissimus dorsi* muscles at five temperature incubations to 6 h post mortem.

SDS-PAGE (Fig.2) shows that incubation at 40 °C reduced bands distinctly at the position of phosphorylase (97 kDa) and creatine kinase (44 kDa) in the sarcoplasmic protein fraction. A corresponding increase of the 97 kDa band in the myofibrillar protein fraction was observed, whereas the 44 kDa band co-migrated with actin (43 kDa) in the gels. Western blot (Fig. 3) confirmed that phosphorylase and creatine kinase translocated to the myofibrillar fraction at 40 °C. Storage to 72 h post mortemdid not result in additional protein translocation.

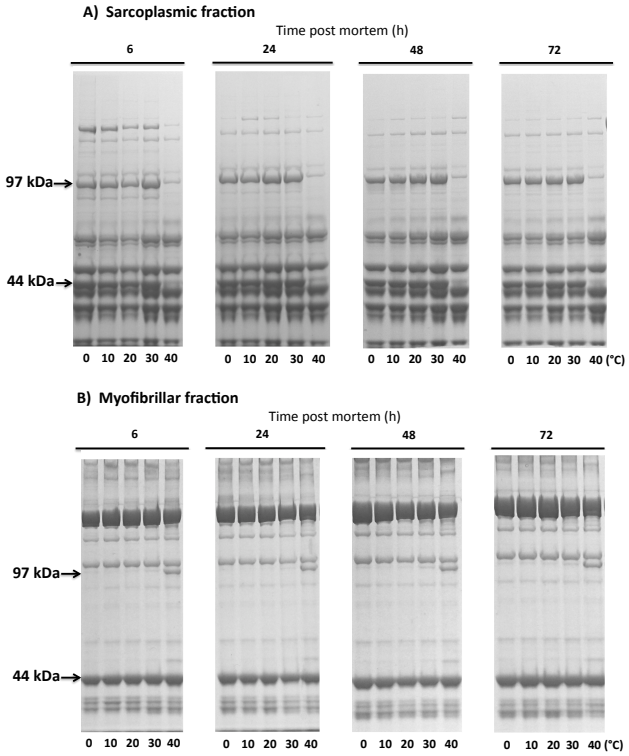

Figure 2. SDS-PAGE of sarcoplasmic and myofibrillar fraction proteins extracted from *Longissimus dorsi* muscles incubated at five temperatures to 6 h post mortem.

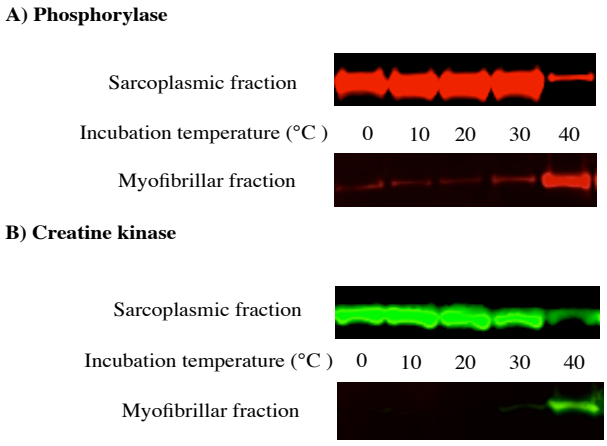

Figure 3. Western blot of glycogen phosphorylase and creatine kinase from myofibrillar and sarcoplasmic fraction. Only 72 h post mortem is shown.

The current study shows that enzyme translocation was paralleled with higher drip loss at 40 °C than in other temperatures ($p<0.05$) (Table 1). This supports the idea that high temperature incubation pre-rigor could induce PSE-like properties, including reduced water-holding and denaturation

of the enzymes phosphorylase [5] and creatine kinase [7].

Table 1. Least square means of meat quality measurements of *Longissimus dorsi* muscles in five temperature groups.

Measurement		Temperature (ºC)				
		0	10	20	30	40
Accumulated drip[1)] (%)	24 h	0.5^{by}	0.5^{by}	0.3^{by}	3.8^{by}	12.0^{a}
	48 h	4.2^{bxy}	4.3^{bxy}	3.5^{bxy}	6.8^{bxy}	13.9^{a}
	72 h	7.5^{bx}	7.7^{bx}	7.1^{bx}	9.9^{bx}	15.8^{a}
Allo-Kramer shear force (N/g)	72 h	142^{a}	128^{ab}	110^{ab}	147^{a}	88^{b}
Sarcomere length (µm)	24 h	1.36^{y}	1.56^{y}	1.48^{y}	1.45^{y}	1.39^{y}

[abc]LSmeans with the same letter within each row do not differ ($p > 0.05$);
[xyz]LSmeans with the same letter within each column do not differ ($p > 0.05$);
[1)] Accumulated drip loss was calculated by (weight without drip [24 h, 48 h, 72 h]-initial weight)/ initial weight x 100%.

A high temperature (of around 40 °C) early post mortem in combination with a pH approaching the rigor level of 5.5 can result in both sarcoplasmic and myofibrillar protein denaturation, which is believed to reduce the water retained by myofibrillar proteins within meat [2,8]. Hamm [10] concluded that water-holding capacity is determined by the electrostatic forces between the filaments. Accordingly, treatments resulting in less repulsion will shrink the myofibrils laterally and thereby reduce the water-holding. The sarcoplasmic proteins that denatured during high temperature incubation in the present study may have precipitated onto myofibrils, resulting in shielding of the negative charges on the myofilaments. Consequently the myofibrils shrink due to the less repulsion. Offer and Knight [8] and Puolanne and Halonen [10] stressed the importance of protein surface/sarcoplasm interactions, which may be influenced by protein coagulation on myofilaments as well.

The substantial tenderization of meat from the 40 ºC group (Table 1) may be due to proteolysis. Previous studies indicate that fast pH decline early post mortem results in a loss of the activity of native µ-calpain in pork [11,12]. In agreement the present study showed at 40 ºC a significantly faster loss of native µ- and m-calpain activity in accompany with the fast pH drop rate (Fig.4).

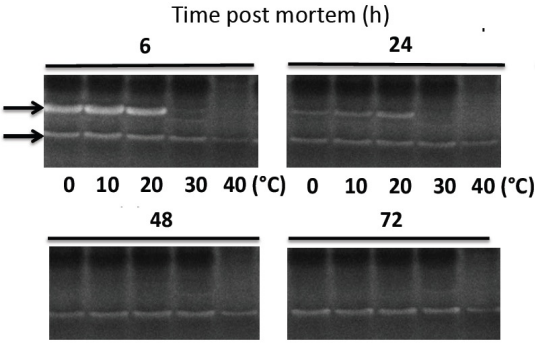

Fig.4 Zymograms showing activity of native µ-calpain (upper arrow) and m-calpain (lower arrow) extracted from *Longissimus dorsi* muscles incubated at different temperatures to 6 h post mortem

Free calcium in the sarcoplasm can increase due to high temperature, and together with low pH induce the autolysis of both µ- and m-calpain [13]. Autolysis reduces the stability, making autolyzed calpains susceptible to loss of activity post mortem [14]. The low level of extractable µ- and m-calpain following incubation at 40 ºC (Fig. 3) may therefore be due to activation by increased calcium and subsequent autolysis and loss of native activity, in turn suggesting that increased proportion of activated m-calpain at 40 ºC account for the observed increase in meat tenderness (Table 1).

IV. CONCLUSION

In conclusion, our study shows that in pork the fast glycolysis induced by elevated temperature (40 °C) early post mortem causes the muscle to go through a combination of low pH (5.5) and high temperature. During this process phosphorylase and creatine kinase translocate from sarcoplasm to myofibril fraction, suggesting their precipitation onto myofilaments. Precipitated denatured protein shielding charged groups on myofilaments can be the causative factor for the poor post-rigor water-holding capacity in meat exposed early post mortem to the combination of high temperature and low pH. Furthermore, reduced activity of the

extractable μ-calpain and m-calpain was paralleled with an increase in tenderness when muscle was exposed to an elevated temperature (40 °C).

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