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Effect of pre-rigor temperature incubation on sarcoplasmic protein solubility, calpain activity and meat properties in porcine muscle

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ABSTRACT

The aim of the study was to investigate the effect of pre-rigor temperature incubation on sarcoplasmic protein characteristics in relation to meat properties within porcine muscle. Porcine *Longissimus dorsi* muscles were incubated at temperatures of 0, 10, 20, 30 and 40 °C to 6 h post mortem. Incubation at 40 °C induced a significant decrease of sarcoplasmic protein solubility and an increase in proteins in the myofibrillar fraction. The protein relocation was followed till 72 h post mortem but had largely been completed by the end of the temperature incubation at 6 h. SDS-PAGE and Western blot analyses suggested that phosphorylase and creatine kinase precipitated onto the myofilaments during incubation at 40 °C. Drip loss increased following incubation at 40 °C, indicating that the precipitation of phosphorylase and creatine kinase may be a factor of reduced water-holding capacity at the combination of high temperature and low pH. Incubation at 40 °C 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 at high temperatures and low pH early post mortem.

1. Introduction

It is generally agreed that PSE (pale, soft, exudative) meat development is a result of abnormally fast glycolysis post mortem. The muscle attains low pH while the muscle temperature is still high (Bendall & Wismer-Pedersen, 1962; Briskey & Wismer-Pedersen, 1961). The combination of high temperature and low pH is able to induce protein denaturation, which is a decisive factor of reduced water-holding capacity of meat (Fischer, Hamm, & Honikel, 1979; Offer & Trinick, 1983; Van Laack & Kauffman, 1999). The denaturation of the sarcoplasmic proteins is believed to be linked to the poor water-holding capacity of PSE meat (Bendall & Wismer-Pedersen, 1962; Joo, Kauffman, Kim, & Park, 1999). The loss of solubility of sarcoplasmic proteins has been used as an indicator for their denaturation (Bendall & Wismer-Pedersen, 1962; Joo et al., 1999; Warner, Kauffman, & Greaser, 1997). Both Warner et al. (1997) and Joo et al. (1999) reported the sarcoplasmic and myofibrillar protein solubility of PSE, RSE (reddish-pink, soft, exudative), RFN (reddish-pink, firm, exudative) and DFD (dark, firm, dry) loins, and observed that the sarcoplasmic protein solubility showed higher correlation to water-holding capacity than that of the myofibrillar proteins. The sarcoplasmic proteins phosphorylase and creatine kinase showed reduced solubility and disappeared from the sarcoplasmic fraction of PSE meat (Joo et al., 1999; Ryu, Choi, & Kim, 2005; Warner et al., 1997).

PSE-like pork can be produced experimentally by pre-rigor incubation of *Longissimus* muscle at 35 °C for 7 h followed by chilling (Lesiów & Xiong, 2013). Incubation of pre-rigor muscle at 37 °C for 4 h post mortem has induced PSE meat characteristics in beef (Scopes, 1964) and pork (Borchert, Powrie, & Briskey, 1969) with decreased intensity of the creatine kinase band in the sarcoplasmic fraction following starch gel electrophoresis. Fischer et al (1979) reported that incubation of post-rigor porcine muscles at 40 °C for 90 min reduced the solubility and activity of glycogen phosphorylase to a similar level as that of PSE pork. Also in poultry it has been reported that pre-rigor incubation of *Pectoralis major* muscles at a temperature of 40 °C induced phosphorylase denaturation together with increased drip loss in broiler chicken (Zhu, Ruusunen, Gusella, Zhou, & Puolanne, 2011) as well as in turkey (Zhu et al., 2013). The precipitation of sarcoplasmic proteins in PSE and PSE-like conditions is thus well documented, whereas information on when and where post mortem the protein precipitation occurs is lacking. The carcass chilling rate plays a great role for the ultimate meat quality and influences a range of biochemical and structural processes such as cold- and heat shortening, proteolysis and meat tenderization. Pomponio and Ertbjerg (2012) studied how the calpain system was affected by prolonged exposure of temperatures in the range 2–30 °C. However, the effect of temperature in the pre-

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rigor period on calpain activity has not been elucidated. Therefore we induced differences in glycolysis rate by pre-rigor incubation of porcine *Longissimus dorsi* muscles at 0, 10, 20, 30 and 40 °C. The aim was to study the effect of pre-rigor temperature incubation on sarcoplasmic protein characteristics particularly in the subsequent storage period and the relation to meat quality within porcine muscle. The protein solubility of phosphorylase and creatine kinase as well as calpain activity and meat quality parameters were investigated in the period from 6 to 72 h post mortem. The results have implications for the control of early pre-rigor temperature in industry as well as knowledge of water-holding capacity in meat.

2. Materials and methods

2.1. Sample collection and pre-rigor incubation

Six porcine *Longissimus dorsi* muscles were obtained randomly from four pig carcasses. Pigs were slaughtered at HK-Ruokatalo, Forssa Slaughterhouse, and muscles were collected at two different days. Each *Longissimus dorsi* muscle was excised at 45 min post mortem and cut into five pieces. Each of them was then divided into two parts; one for drip loss measurements and another for protein analysis. Muscle pieces were weighted and sealed in polyethylene bags for incubation in water bath at temperatures of 0, 10, 20, 30 and 40 °C (Temperature incubation) from approximately 50 min post mortem until 6 h post mortem. After the incubation samples were kept on ice during 2 h transport from the slaughterhouse to the laboratory, thereafter bags were hung in the cold room (4 °C) for drip loss measurement until 72 h post mortem. Bags for protein analysis were stored at the same temperature for sampling at 24, 48 and 72 h.

2.2. Physical measurements

Temperature and pH were measured before incubation (50 min post mortem). During the temperature incubation, polyethylene bags were taken out at 2, 3, 4 and 6 h for pH and temperature measurements. Temperature was measured in the center of the samples by a Microtherma 2 thermometer (E. T. I. LTD, Worthing, UK). pH was measured from homogenates prepared by homogenizing 0.4–0.5 g of meat with 10 volumes of 5 mmol/L Na-Iodoacetic acid and 150 mmol/L KCl by using a Knick Portamess 752 pH-meter equipped with a Mettler-Toledo Inlab 427 electrode. The ultimate pH was measured at 24 h post mortem (Jeacocke, 1977).

2.3. Meat quality measurements

Sarcomere lengths were measured at 2, 4, 6 and 24 h post mortem. About 1 g of meat was mixed with formalin solution (35 g/L formaldehyde in 85 mmol/L phosphate buffer). Thereafter the samples were homogenized with Potter–Elvehjem-Type Tissue Grinder with PTFE Pestle (Tomas Scientific, Swedesboro, NJ). One drop of homogenate was pipetted onto a glass slide, smeared and placed under Novette 1507-0 Helium-neon gas laser (Uniphase, Manteca, CA). The sarcomere length was determined by observing the light diffraction pattern of myofibrils according to Cross, West, and Dutson (1981). An average value was obtained based on 20 determinations from each sample.

For drip loss measurements, a gravimetric method was used (Honikel, 1998). At 24, 48 and 72 h, the bags were opened and the exudate on the surface was carefully removed before the meat pieces were weighted again. The percentage of drip loss was calculated as the accumulated drip in the storage period by taking the weight at each time point post mortem (24, 48 and 72 h) compared to the initial weight. After measuring the drip loss at 72 h, the meat pieces were vacuum packaged and cooked at 77 °C for 45 min. The core temperature was recorded by a Microtherma 2 thermometer, obtaining values of 74.0–76.5 °C. After cooking, meat packages were stored at 4 °C overnight. The next day meat pieces were weighted after removing the liquid on the surface. The decrease in weight as a percentage of the weight before cooking was taken as cook loss. For shear force measurements, the same meat piece after cooking was used. From each peace 4 to 5 cubes of 20 × 20 × 6 mm (fiber axis along 20 mm direction) were cut and weighted. Then each cube was placed in an Allo-Kramer shear cell and cut across the fiber axis using an Instron Model 6625 (Instron Co, Canton, MA). The average value was calculated as the shear force (N/g) of each meat piece.

2.4. Protein analysis

2.4.1. Protein extraction

Muscle pieces for protein analysis were sampled at 6, 24, 48 and 72 h post mortem and frozen in liquid nitrogen. Homogenates were prepared using a slightly modified method from Joo et al. (1999). Two grams of the frozen meat was homogenized by IKA Ultra-Turrax T25 homogenizer (Labortechnik, Staufen, Germany) at 13,500 rpm for 20 s in 40 mL cold rigor buffer (75 mmol/L KCl, 10 mmol/L KH_2PO_4, 2 mmol/L $MgCl_2$, 2 mmol/LEGTA, pH 7.0). Samples were run in duplicates. Then the homogenate was centrifuged at 10,000× *g* at 4 °C for 10 min. The supernatant was collected as the sarcoplasmic fraction and kept on ice. Twenty mL cold rigor buffer was added to the pellet and it was then homogenized again with the same homogenizer at same speed for 10 s and centrifuged at 10,000× *g* at 4 °C for 20 min to wash the myofibrils free of sarcoplasmic proteins. The pellet, which was mainly myofibrils, was re-suspended by homogenization in 20 mL cold rigor buffer for 10 s. Then the protein content was determined by RC DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA). Results calculated from the average of two duplicates were taken as protein content.

2.4.2. SDS-PAGE

After adjusting the homogenates to the same protein concentration, 32 μL of diluted homogenate was mixed with 12.5 μL NuPAGE® LDS Sample Buffer (4X) and 5 μL NuPAGE® Sample Reducing Agent (10X) (Invitrogen, Carlsbad, CA). Then the mixture was heat treated at 100 °C for 3 min and applied onto 7% Tris-Acetate gels (Invitrogen). A standard was prepared from the sarcoplasmic fraction of meat at 6 h post mortem incubated at 10 °C. The standard was loaded 3 times on each gel. Gels were settled in XCell SureLock® Mini-Cell electrophoresis chamber and the electrophoresis was run at 150 V for approximately 90 min. Gels were after staining (400 mL/L ethanol, 100 mL/L acetic acid and 10 g/L Coomassie Blue R-250) and distaining (100 mL/L ethanol and 75 mL/L acetic acid) placed on a light board and pictured by a digital camera.

2.4.3. Western blot

After electrophoresis, proteins in gels were transferred to Immobilon-FL Transfer Membrane (Millipore, Bedford, MA) in XCell II™ Blot Module with NuPAGE® Transfer Buffer (20X) from Invitrogen. The blotting process was performed for 1 h at 30 V. After blotting, membranes were blocked for 1 h in 20 mL of TBS (50 mmol/L Tris, 150 mmol/L NaCl, pH 7.5) with 50 g/L skim milk powder at room temperature. Then membranes were washed in TBST (50 mmol/L Tris, 150 mmol/L NaCl, 0.5 g/L Tween-20, pH 7.5) for 10 min. Membranes were then incubated with two primary antibodies at the same time: 10 μL rabbit polyclonal anti-glycogen