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Influence of early pH decline on calpain activity in porcine muscle

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ABSTRACT

This study investigated the influence of post-mortem pH decline on calpain activity and myofibrillar degradation. From 80 pigs, 30 Longissimus dorsi (LD) muscles were selected on the basis of pH values at 3 h post-mortem and classified into groups of 10 as fast, intermediate and slow pH decline. The rate of pH decline early post-mortem differed between the three groups, but the ultimate pH values were similar at 24 h. Calpain activity and autolysis from 1 to 72 h post-mortem were determined using casein zymography and studied in relation to myofibrillar fragmentation.

Colour and drip loss were measured. A faster decrease in pH resulted in reduced level of μ-calpain activity and increased autolysis of the enzyme, and hence an earlier loss of activity due to activation of μ-calpain in muscles with a fast pH decline. Paralleling the μ-calpain activation in muscles with a fast pH decline a higher myofibril fragmentation at 24 h post-mortem was observed, which was no longer evident in the later phase of the tenderization process. In conclusion, the rate of early pH decline influenced μ-calpain activity and the rate but not the extent of myofibrillar degradation, suggesting an early effect of proteolysis on myofibril fragmentation that is reduced during ageing due to an earlier exhaustion of μ-calpain activity.

1. Introduction

Meat tenderness is one of the most important factors to consumers (Shackelford et al., 2001), and it is well established that post-mortem proteolysis improves meat tenderness. One of the protease systems that have been shown to be involved in the degradation of muscle protein is the calpain system (Huff-Lonergan & Lonergan, 2005; Koohmaraie, Seideman, Schollmeyer, Dutson, & Crouse, 1987). The calpain system consists of several proteases, and two members, μ- and m-calpain and their inhibitor calpastatin, are thought to be involved in the proteolytic tenderization of meat. Both μ- and m-calpain are calcium dependent and are composed of a 80 and a 28 kDa subunit, both autolyze in presence of calcium (Cong, Goll, Peterson, & Kapprell, 1989). The 80 kDa subunit, the catalytic part, is reduced to a 76 kDa form by autolysis (Goll, Thompson, Li, Wei, & Cong, 2003), which reduces its calcium requirement for activity (Goll, Geesink, Taylor, & Thompson, 1995). In addition to calcium concentration, other parameters like temperature and pH influence the activity of calpain (Camou, Marchello, Mares, Vazquez, & Goll, 2006).

Early post-mortem pH development plays an important role in determining the rate and extent of muscle tenderization (O'Halloran, Troy, Buckley, & Reville, 1997a) and the colour characteristic of meat (Lindahl, Henckel, Karlsson, & Andersen, 2006). Many studies analysed the influence of early pH development on the tenderness of meat, mostly related to beef (O'Halloran, Troy, & Buckley, 1997b; O'Halloran et al., 1997a). In these studies, the rate of pH decline was influenced using electrical stimulation (Martin, Murray, Jeremiah, & Dutson, 1983), chilling treatments (Whipple, Koohmaraie, Dikeman, & Crouse, 1990; White, O'Sullivan, O'Neill, & Troy, 2006) or a combination of both (Hwang & Thompson, 2001; Rhee, Ryu, & Kim, 2006); all the experiments showed a relationship between rate of pH decline and meat tenderness. However, the precise mechanism of interaction between post-mortem pH changes and tenderness is not known.

The influence of pH decrease on pork characteristics has been studied, especially in Halothane Genotype (Offer, 1991; Tam et al., 1998) and looking at the influence of PSE and pH on drip loss (Offer, 1991) and colour (Lindahl et al., 2006; Tam et al., 1998). Faster decrease in pH after slaughter leads to a lighter colour of pork (Huff-Lonergan et al., 2002; Lindahl et al., 2006; Tam et al., 1998) and higher drip loss in meat (Bee, Anderson, Lonergan, & Huff-Lonergan, 2007; Bertram, Whittaker, Andersen, & Karlsson, 2003; Martin et al., 1983; Offer, 1991).

Several studies have indicated that a fast decrease in pH post-mortem is associated with a faster decrease of μ-calpain activity (Hwang & Thompson, 2001; Rhee et al., 2006; Whipple et al., 1990) and a low μ-calpain activity was detected in muscles with lower pH post-mortem (Claeys, De Smet, Demeyer, Geers, & Buys,

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Table 1
pH and temperature decrease in Longissimus dorsi muscle during the first 72 h post-mortem.

	Slow pH decline (pH 3 > 6.30)	Intermediate pH decline (6.00 < pH 3 > 6.30)	Fast pH decline (pH 3 < 6.00)
Muscle (pH)			
1 h	6.70[a,x]	6.57[b,x]	6.28[c,x]
3 h	6.48[a,y]	6.19[b,y]	5.72[c,y]
6 h	6.20[a,z]	5.87[b,z]	5.57[c,z]
24 h	5.52[w]	5.51[w]	5.45[w]
72 h	5.45[w]	5.50[w]	5.46[w]
SEM	0.040	0.040	0.040
Muscle temperature (°C)			
1 h	38.74[x]	38.78[x]	39.62[x]
3 h	25.52[y]	24.80[y]	26.89[y]
6 h	17.52[z]	17.33[z]	17.67[z]
24 h	4.99[w]	5.27[w]	5.14[w]
72 h	4.42[w]	4.81[w]	4.95[w]
SEM	0.427	0.427	0.427

[a,b,c] Within rows, mean values without a common superscript differ ($p < 0.05$).
[x,y,z,w] Within columns, mean values without a common superscript differ ($p < 0.05$).

2001; Uytterhaegen, Claeys, & Demeyer, 1992). In contrast, O'Halloran et al. (1997a) found a higher μ-calpain activity in fast glycolysing muscle. Different rates of pH decrease also influence the degradation of muscle protein, although it is not clear whether a faster degradation of proteins is associated with a fast pH decline (O'Halloran et al., 1997b, 1997a; Uytterhaegen et al., 1992) or with a slow pH decline (White et al., 2006).

The detection of autolyzed calpain in muscle and meat is believed to be indicative of proteolytic activity (Geesink & Koohmaraie, 1999; Goll et al., 2003; Melody et al., 2004). Comparing different porcine muscle it was suggested that the rate of pH decline influences the rate of μ-calpain autolysis and that a faster pH decline parallels an earlier appearance of the autolyzed form of the enzyme (Melody et al., 2004). Concomitant with an earlier appearance of the autolyzed enzyme, measured as the appearance of the 76 kDa proteolytic form of μ-calpain, an earlier degradation of myofibrillar proteins like desmin and titin was determined (Melody et al., 2004; Zhang, Lonergan, Gardner, & Huff-Lonergan, 2006). The aim of the present work was to study the influence of post-mortem pH decline in pig LD muscle in relation to calpain activity and myofibrillar degradation.

2. Materials and methods

2.1. Samples descriptions

Eighty Italian Duroc × (Landrace × Large White) cross bred pigs, mainly female with an average live weight of 162 ± 12 kg, pen-fed and finished in northern Italy under standard Parma-ham practice, were transported to a local slaughter house in the same truck and slaughtered the following day. After slaughter, the carcasses were divided into cuts and kept at 18 °C until 3 h post-mortem. Thereafter, the loins were stored in a chilling room at 2 °C. In loins, including vertebras and ribs, pH was measured (Eutech Instruments pH 6) at 1 (pH 1) and 3 h post-mortem (pH 3) at the 7th rib of the right side of the carcass. Based on pH 1 and pH 3 values, 30 pigs were selected and right side loins were divided into three groups of 10 on the basis of the rate of the pH decline post-mortem: fast pH decline (pH 3 < 6.00), intermediate pH (pH 3 in the interval 6.00–6.30) and slow pH decline (pH 3 > 6.30) (Table 1). To avoid PSE, animals with low pH 1 (pH 1 < 5.70) were discharged. The pH electrode was calibrated at the appropriate carcass temperature for each time point. The pH and temperature (digital thermometer TFA DT 90) of the LD muscle were monitored at 1, 3, 6, 24 and 72 h post-mortem. Colour measurements (lightness L^*, redness a^* and yellowness b^*) were recorded at 4.5, 6, 24, 72 and 144 h post-mortem using a Minolta CRC 300 instrument (Minolta Co., Tokyo, Japan) with illuminant D65 and calibrated against a white plate ($Y = 93.0$, $x = 0.3162$, $y = 0.3322$). Samples for calpain activity were taken 1 and 6 h post-mortem at the 8th rib and immediately frozen in liquid nitrogen and stored at −80 °C overnight in the chilling room. The next day, LD were deboned, placed in vacuum bags and transported, covered by dry ice and stored in isolated boxes, to the University of Bologna and stored at 4 °C. Muscles were sampled for calpain activity at 24 h and 3 d and myofibril fragmentation at 24 h, 3 d and 6 d post-mortem and frozen at −80 °C. For drip loss determination, at day 1 a 5 cm slice was isolated, weighed, placed in a commercial tray and covered with a plastic film and stored at 4 °C. After 2 days (72 h post-mortem) the samples were blotted with paper and reweighted. Drip loss was calculated as percent fluid loss. Frozen samples were transported to the University of Copenhagen in dry ice before myofibril fragmentation and calpain activity measurements.

2.2. Myofibril fragmentation

The myofibril fragmentation was measured as described (Lametsch, Knudsen, Ertbjerg, Oksbjerg, & Therkildsen, 2007). Briefly, samples of 2.5 g were homogenized in 30 mL cold buffer (100 mM KCl, 20 mM potassium phosphate, 1 mM EGTA, pH 7.00) at 20,500 rpm using an Ultra-Turrax T25 equipped with an S25 N-18 G dispersing element (Ika Labortechnik, Staufen, Germany). Samples were analysed in duplicates and sizes of myofibrils were determined as surface mean diameter D (3,2), measured using a Malvern Mastersizer Micro Plus (Malvern Instruments Ltd., Worcestershire, UK). The distribution in the size of the myofibrillar fragments was determined as surface mean diameter D (3,2).

2.3. Extraction procedure for calpain determination

From each sample, frozen at −80 °C, duplicates of 1 g meat were homogenized using an Ultra-Turrax T25 Mixer (13,500 rpm) in 6 mL of an extraction buffer (100 mM Tris; 5 mM EDTA; 10 mM monothioglycerol; one tablet per 50 mL of Protease Inhibitor Cocktail Tablet COMPLETE, RAS Roche Applied Science, Mannheim, Germany; pH 8.0) and centrifuged for 30 min at 4 °C with a speed of 15,000*g*. An aliquot of the supernatant was mixed with glycerol to a final concentration of 30% and stored at −80 °C until analysed for calpain activity.

2.4. Casein zymography method

Calpain activity was measured using a casein zymography method as described by Pomponio et al. (2008) with some modification. Each duplicate of the samples was run in triplicate using 12.5% casein precast gels (Bio-Rad Laboratories, Hercules, CA). One volume of sample buffer (300 mM Tris, 40% glycerol, 0.02% bromophenol blue, 100 mM DTT, pH 6.8) was mixed with 3 volumes of supernatant and the sample was loaded on the gel. The electrophoresis was carried out at 80 V for 3 h at 4 °C (running buffer: 25 mM Tris, 192 mM glycine, 1 mM EDTA, pH 8.3). The gels were incubated with shaking at room temperature in 100 mL incubation buffer (50 mM Tris, 4 mM $CaCl_2$, 10 mM monothioglycerol, pH 7.5) for 1 h, changing the buffer after 30 min. The calpain activity was stopped by washing the gel for 30 min with shaking, using 20 mM Tris, 10 mM EDTA, pH 7.0. The gels were stained overnight with colloidal Coomassie brilliant blue G and destained the next day with frequent change of deionised H_2O. The size of the clear area denoting calpain activity was measured with LabScan