

# TENDERISATION OF PORK AS AFFECTED BY DEGREE OF COLD INDUCED SHORTENING

IVERSEN P.\*\*\*, HENCKEL P.\*\*\*\*, LARSEN L.M.\*\*, MONLLAO S.\*\* and MØLLER A.J.\*

\* Department of Dairy and Food Science, \*\* Chemistry Department, \*\*\* Center for Food Research, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark \*\*\*\* Research Centre Foulum, Tjele, Denmark

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## SUMMARY

Porcine M. longissimus dorsi from 12 carcasses were obtained at various times of boning to study the effect of different ageing periods on cold shortened muscle. At one hour and six hours post stunning samples from the right side of each carcass were excised and immediately placed in icewater until 24 hours post stunning. From each time of boning, samples were then allocated by random for storage at -20°C at 24 hours post stunning or aged for seven days at 2°C before stored at -20°C.

Time of boning significantly effected tenderness resulting in decreasing WB shear force values with increasing boning time. Delaying boning time from one to six hours reduced cold induced shortening from 27.4% to 6.1% and concomitantly, the effect of ageing increased. Tenderness improvement for the one hour excised cuts was only 7.3% as compared to 24.5% for samples boned at six hours post stunning. While muscle shortening in early excised cuts was highly affected by boning time, no significant correlation linking muscle shortening and sarcomere length appeared from this study.

## Introduction

The chilling time of pig meat is of great interest to the industry. Reducing the chilling time would be beneficial in reducing evaporative loss, drip loss and the growth of spoilage organisms (Dransfield et al., 1991). Rapid chilling has also been recommended as a way of reducing quality problems associated with PSE pork. On the other hand, toughening caused by rapid chilling (James et al., 1983), particularly in slow glycolysing muscles (Møller & Vestergaard, 1987) is a great risk. However, contradictory results have been reported, Laack & Smulders (1991) found no relationship between rapid chilling rate and toughening in pork.

The process of cold shortening has hitherto been regarded as irreversible, but recent studies have shown an increase in sarcomere length in cold shortened muscle post rigor, indicating a possible reversibility of the actomyosin complex (Feldhusen & Kühne, 1992) supporting the results found by Laack & Smulders (1991).

A further expansion of knowledge within this area has been reported by Møller & Jensen (1993), who investigated cold toughening in pork as effected by time of boning. Although degree of toughness was related to both pH and R-value, the R-value, an indicator for residual ATP, showed the highest predictive value of estimating the risk for cold shortening in early excised cuts.

In this study we looked at different degrees of cold induced shortening and a possible ageing effect in tenderness related to the degree of cold shortening. Various degrees of muscle shortening were obtained in excised pork loins by using different times of boning. Tenderness were evaluated before and after an ageing period of 7 days.

## Materials and methods

### Experimental design and sampling procedure

A group of 12 crossbred pigs at 90 kg-liveweight (Danish Landrace x Duroc x Yorkshire) were stunned by CO<sub>2</sub> and following conventional slaughter procedure, carcasses were held at 12-14°C for 90 min before further chilling at 2°C until 24 h post stunning.

Left sides of the carcasses were used as controls, i.e., M longissimus dorsi (LD), 12-15 rib, was excised at 24 h post stunning and cut into subsamples of meat blocks (20x20x50 mm), the longest dimension being parallel with the muscle fibres. The total number of meat blocks (8 per side) were randomly divided into two groups and vacuumpacked for ageing in 0 or 7 days at 2°C before storage at -20°C for 8-12 weeks before further measurements.

The LD muscle from right side of carcasses was used for early boning. From similar location as used for controls, LD samples were excised at 1 h and 6 h post stunning and cut into subsamples as described for controls, placed into polyethylene bags for chilling in icewater until 24 h post stunning. As for controls, subsamples were vacuumpacked before ageing 0 or 7 days, then stored at -20°C until further measurements.

Furthermore, a 5g meat sample located at the 15th rib was taken at 1 h and 6 h post stunning from right sides and immediately frozen in liquid nitrogen. These samples were further stored at -70°C (8-12 weeks) and used for R-value analysis as described below.

### Temperature and pH

pH was measured in the centre of the LD muscle from the left side carcasses (12-15th rib) at 1, 3, 6, 9 and 24 h post stunning using a direct insertion probe electrode (Ingold Lot 406-M3). At the same intervals and at the same location, temperature was measured using a Technoterm 1500.

### R-value

The samples taken at 1 h and 6 h post stunning and frozen at -70°C were without thawing immediately analysed. The R-value procedure was performed according to Honikel & Fischer (1977).

### Muscle shortening

Small nails were inserted with a distance of 40 mm in meat blocks from samples boned at 1 h and 6 h post stunning. By measuring the distance between nails after chilling at 24 h post stunning, the percentage of muscle shortening was calculated. Four blocks were used at every sampling time and mean values calculated.

### Sarcomere length

From one subsample from each time of boning a 1-cm thick cross-section was cut and one 1-cm<sup>3</sup> cube was prepared. Two sections from the cube were then cut with a thickness of 50 µm longitudinal to the orientation of fibres. The sections were placed on a glass slide and covered with a cover slip. To measure sarcomere length a helium-neon laser with a wavelength of 632.8 nm was used (Weber et al, 1988). Five measurements were done on each section and average sarcomere length calculated.

### Drip loss, cooking loss and Warner-Bratzler shear force

Subsamples (3 blocks in each polyethylene bag) of meat were taken out of the -20°C freezer and weighed. After storage at 2-4°C for 24 h, the percentage of drip loss was calculated. The blocks were then individually placed in 100 ml glass tubes just covered with 0.9% NaCl solution, and heated in a water bath at 80°C for 25 min. After heat treatment, meat blocks were rinsed in cold tap water, drained and reweighed for calculation of percent cooking loss. The meat blocks were placed at 2-4°C for no longer than 24 h before measurement of Warner-Bratzler shear force. The cooked meat blocks of rectangular cross-section were cut to a dimension of 10x10 mm and sheared at right angles to the fibre axis using a Warner-Bratzler shear blade with a triangular slot cutting edge. To measure the peak shear force (N/cm<sup>2</sup>), an Instron Universal testing machine model 4301 was used. On each meat block three peak shear force values were determined giving a total of 9 measurements for calculating mean values from each time of sampling.

### Statistical analyses

Data was analysed by ANOVA with the GLM procedure (unbalanced data) of SAS for a factorial model with 3 factors: Different pigs, time of boning and ageing. Linear correlation (Pearson correlation coefficients) between several variables was performed using the correlation procedure of the SAS programme. Mean values were analysed with Students t-test.

## Results and discussion

The temperature in the loin from controls was at 1 h post stunning 36°C declining to 5°C at 10 h and 2°C after 24 h post stunning. The decline in pH resulted in a pH range from 5.65-5.90 at 6 h post stunning. We found a significant ( $P < 0.001$ ) increase in R-value as time of boning was delayed from one to six hours post stunning showing the diminution of energy (ATP) in the muscle. Compared to other studies the pH declines were similar to the one of conventional chilling and normal preslaughter conditions reported by Gigiel & James (1984) but somewhat faster than the one obtained by Feldhusen & Kühne (1992). Also the R-values at one hour indicated normal meat, since they all were below 1.05 (Honikel & Fischer, 1977).

Cold induced muscle shortening in pre rigor excised LD muscle from pork has previously been reported to be 34% (Dransfield & Lockyer, 1985). From this study, 27% muscle shortening was found in cuts excised at 1 h post stunning, while delaying boning to 6 h post stunning reduced cold shortening markedly to only 6%. Muscle shortening increased drip loss (Table 1) as measured after frozen storage ( $r = 0.38$ ,  $P < 0.05$ , Table 3), while cooking loss was unaffected by the degree of shortening. These results are in agreement with previous work by Honikel et al. (1986), who showed that cooking loss but not drip loss was independent of shortening. The level of drip loss, 10-15% (Table 1), was similar to that found by Irie & Swatland (1993), who also used frozen samples, but higher than normally found on non-frozen meat (Dransfield et al., 1991) showing the decrease in water-holding capacity caused by freezing.

Surprisingly, the marked reduction in muscle shortening from 27.4% to 6.1% in excised muscles as effected by time of boning was not reflected in length of sarcomeres (1.69  $\mu\text{m}$  and 1.69  $\mu\text{m}$  respectively, Table 1).

Tenderness was affected by both time of boning and ageing as measured by Warner-Bratzler (WB) shear force (Table 2). According to our experience, WB values  $> 60 \text{ N/cm}^2$  are unacceptable as determined by taste panels. The cuts excised at 1 h post stunning and then exposed to icewater were extremely tough (96.4  $\text{N/cm}^2$ ), and ageing reduced toughness only by 7.3%. The cuts excised at 6 h post stunning were also tough (77.9  $\text{N/cm}^2$ ), but here it's noteworthy that the effect of ageing reduced toughness by 24.5% and resulted in acceptable WB shear force values (58.8  $\text{N/cm}^2$ ). Boning at 24 h (controls) produced WB shear force values of 56.3  $\text{N/cm}^2$ , and ageing increased tenderness by 12.5%.

Although muscle shortening was markedly reduced by delaying boning to 6 h post stunning, those samples showed considerable cold toughening with WB values at 77.9  $\text{N/cm}^2$ . For the unaged meat, cold toughening has therefore occurred without severe shortening (6 h excised cuts). WB shear force values decreased due to time of boning and was significantly related ( $r = 0.46$ ,  $P < 0.05$ ) to muscle shortening in agreement with the results reported by Møller & Jensen (1993).

As compared to unaged meat, a large effect of muscle shortening on WB values appeared after a 7 d ageing period. For cuts excised at 1 h post stunning, the tenderisation showed only a minor decrease in WB values, while for the 6 h excised cuts a much larger decrease in WB values appeared. Contradictory results has previously been reported on the effect of ageing in cold shortened pork. Thus, Dransfield & Lockyer (1985) found reduced tenderisation in highly shortened pork, while Feldhusen & Kühne (1992) showed ageing to produce tender meat even at high values of muscle shortening.

When muscle shortening was diminished, improved tenderisation could be due to a higher accessibility for the endogenous proteolytic enzymes (calpains, cathepsins) on the myofibrillar proteins. Both  $\mu$ -calpain and m-calpain have been shown to be active until 7 d post stunning (Iversen et al., 1993).

Further studies seem necessary to improve our knowledge on the mechanisms of ageing related to tenderness, especially to quantify the relative importance of the different enzyme systems as well as the accessibility of enzymes on myofilaments varying in degree of contracture.

## Conclusion

The effect of time of boning on the potential of cold induced shortening was studied. A significant reduction in cold shortening was found, when delaying boning time from one to six hours. Concomitantly, the effect of ageing (7 days) increased from 7.3% for the one hour excised cuts to 24.5% for the six hours excised cuts. Possibly the improved tenderisation with diminished muscle shortening could be due to a higher accessibility of the endogenous proteolytic enzymes.

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Table 1. Mean Values and Standard Deviation of Drip loss (%), Cooking loss (%) and Sarcomere length ( $\mu\text{m}$ ) at different times of boning (n=12).

Table 2. Mean Values and Standard Deviations of WB Shear Force ( $\text{N}/\text{cm}^2$ ) at different times of boning and aged until either 1 d or 7 d post stunning with calculated Ageing Effect (%) in Tenderness (n=12).

Table 3. Coefficients of correlation (r) between tenderness parameters