NMR RELAXOMETRY AND TOUGHNESS CHANGES IN LONG TIME LOW TEMPERATURE (LTLT) TREATED PORK LOIN

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Abstract

In order to elucidate the effect of low temperature, long time (LTLT) heat treatment of pork on tenderness and cooking loss, porcine *longissimus dorsi* samples were heat-treated at 53°C, 55°C, 57°C and 59°C for 3 h and 20 h. Warner-Bratzler Shear Force, cooking loss, sarcomere length and low-field NMR T_2 relaxation time were investigated. Results showed that shear force decreased and cooking loss increased with increasing temperature and time. NMR T_2 relaxation time could divide the treatments according to temperature, with 53°C and 55°C being separated from 57°C and 59°C, indicating that changes occur between 55°C and 57°C. Sarcomere length did not differ between the treatments.

***Index Terms*— cooking loss, low field NMR, sarcomere length, tenderness, Warner-Bratzler Shear Force.**

I. INTRODUCTION

Long term low temperature (LTLT) treatments of meat have been investigated for decades, and several authors have concluded that LTLT treatment result in more tender meat (Machlik & Draudt, 1963; Laakkonen, Wellingt, & Sherbon, 1970; Bouton & Harris, 1981; Vaudagna et al., 2002). However, previous studies have solely been focusing on beef muscles, while the effect of LTLT treatment on pork muscles has not been investigated.

Denaturation of meat proteins upon heating cause structural changes, such as tissue shrinkage, and thereby affect the water within the meat. Low-field NMR have been used to study water distribution in meat. According to Bertram, Wu, van den Berg & Andersen (2006) and Micklander, Peshlov, Purslow & Engelsen (2002) changes in T_2 relaxation times upon cooking of porcine *longissimus dorsi* between 53-58°C were reflecting myosin denaturation (53-58°C) and longitudinal shrinkage of muscle fibres at 57°C.

In a recent study we showed that during LTLT treatment of porcine *longissimus dorsi* a significant decrease in toughness and an increase in cooking loss was observed between 53°C and 58°C (Christensen, Ertbjerg, Aaslyng, & Christensen, 2009). The aim of the present study was therefore to investigate the changes in toughness and water properties at temperatures between 53°C and 58°C in order to obtain a better understanding on water mobility during LTLT treatments.

II. MATERIALS AND METHODS

Raw material

M. longissimus dorsi were excised 24 hours *postmortem* from 6 commercially available slaughterpigs. pH was measured and used as a selection criteria for the muscles (acceptable range pH 5.55-5.80). The muscles were vacuum packed, stored 4 days at 5°C, and were then cut into samples of app. 4x12x10 cm, vacuum packed and frozen at -20°C.

Thermal treatments

Muscle samples were thawed overnight at 4°C and the thermal treatments were carried out in water baths (ICC "Roner", Frinox Aps, Hillerød, Denmark) set at 53°C, 55°C, 57°C and 59°C. Each water bath contained 3 samples at the same time and heat treatments were performed for 3 h and 20 h each. Heat treatments were arrested by keeping the samples in ice water for 10 min. Cooking loss was measured by weighing the samples before and after heat treatment and expressed in percent of original weight. Mean values from 3 repetitions of each thermal treatment were obtained. The samples were then stored overnight at 4°C.

Warner-Bratzler Shear Force
Six blocks of 1x1x6 cm were cut from the heat treated samples and the shear force was measured 3 times on each block by TA-HDi Texture Analyzer (Stable Micro Systems, UK) equipped with a Warner-Bratzler test cell. The mean maximum force required to shear through the sample from 3 repetitions of each thermal treatment was determined.

Sarcomere length
Sarcomere length were measured on samples heat treated to 53°C and 59°C for 3 and 20 h. Samples were frozen at -20°C after heat treatment, and slices of 10µm were cut on Frigocut (AusJena, Germany) and fixed on a glass slide. Sarcomeres were examined with a light microscope equipped with a camera, and sarcomere lengths were measured using the software Image-Pro Plus (Media Cybernetics Inc., Silver Spring, USA).

Low-field NMR
The NMR relaxation measurements were performed on a Maran Benchtop Pulsed NMR Analyzer (Resonance Instruments, Witney, UK) with a resonance frequency for protons of 23.2 MHz. The NMR instrument was equipped with an 18 mm variable temperature probe. The meat strips were cut and placed in cylindrical glass tubes for the NMR measurements and tempered in a 25°C water bath for 15-20 minutes prior to measurement. Transverse relaxation, T_2, was measured using the Carr-Purcell-Meiboom-Gill sequence (CPMG). The T_2 measurements were performed with a τ value (time between 90° pulse and 180° pulse) of 150 µs. The 90° and 180° pulses were 8.2 and 16.4, respectively. The repetition time between two scans was 3 s. Data from 4096 echoes were acquired as 16 scan repetitions, with one dummy scan. Only data from even-numbered echoes were used in further data analysis to avoid influence of imperfect pulse settings. The obtained T_2 relaxation decays were analyzed using distributed exponential fitting analysis (Menon, Rusinko, & Allen, 1991).

Statistical analysis
Analysis of variance was performed using SAS Software 9.2. The model included temperature and time as fixed effects, while animal and sample location were included as random effects. The model used was y = µ + temp + time + temp*time + ANIMAL + LOCATION. In addition, principal component analysis (PCA) was carried out on the obtained NMR T_2 data.

III. RESULTS AND DISCUSSION

The influence of LTLT treatment on NMR T_2 relaxation time is shown in the PCA scoreplot (Figure 1). It is clearly illustrated that samples heated at 53°C and 55°C were separated from samples heated at 57°C and 59°C. Statistic significance was only found between 53°C and 59°C and between 55°C and 59°C, however, a tendency (*P = 0.06*) towards higher T_2 relaxation times at 55°C than at 57°C was observed.

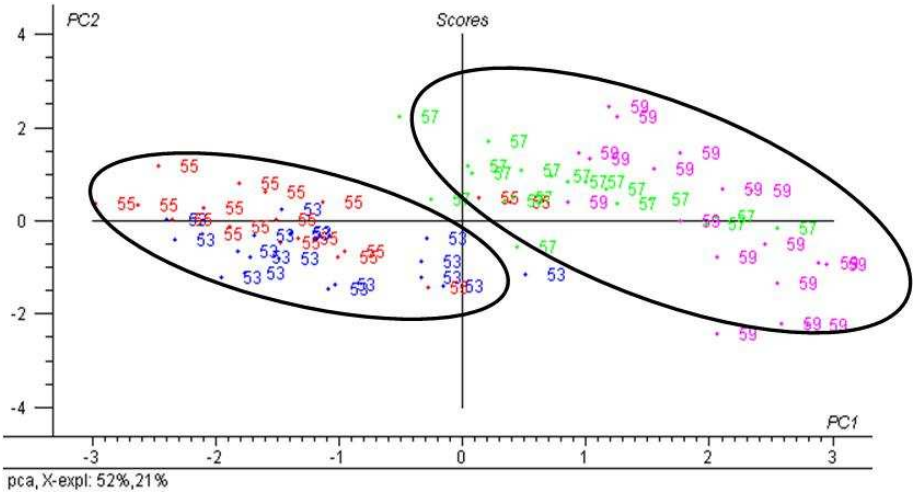

Figure 1. PCA scoreplot (PC1 versus PC2) based on NMR T_2 relaxation times obtained on porcine *Longissimus dorsi* after LTLT treatments at 53°C, 55°C, 57°C and 59°C for 3 and 20 hours.

The percentage of cooking loss after LTLT treatments of pork loin are shown in Figure 2. At 3 hours of cooking, cooking loss was higher (*P < 0.01)* at 57°C and 59°C compared to 53°C and at 59°C cooking loss was higher (*P < 0.01*) compared to 55°C. At 3 hours cooking loss at 55°C and 57°C were identical. At 20 hours cooking loss increased significantly with increasing temperatures, though the amount of cooking losses at 55°C and 57°C were identical. At 53°C the cooking loss was significantly lower, and at 59°C cooking loss was significantly higher compared to the 3

other temperatures.

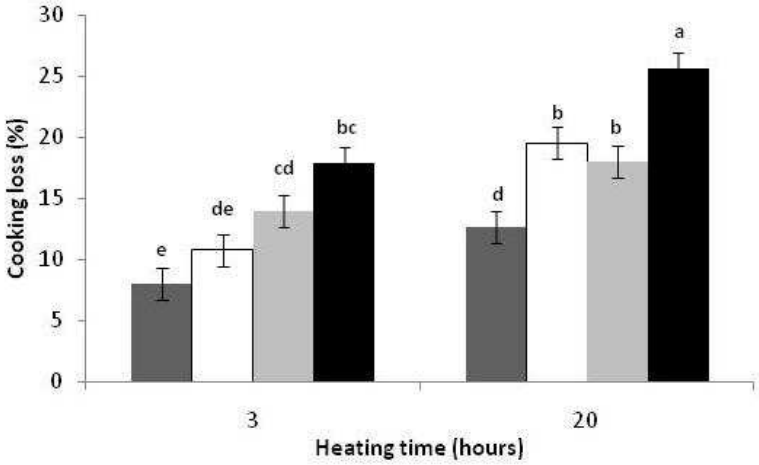

Figure 2. Effect of time and temperature of cooking loss in LTLT treated porcine *Longissimus dorsi*. Dark grey: 53°C; white: 55°C; light grey: 57°C; black: 59°C. Bars represent standard errors (n=3).

Palka & Daun (1999) found a reverse proportionality of sarcomere length and cooking loss, thus cooking loss increased with decreasing sarcomere length during cooking of beef *Semitendinosus* between 55°C and 121°C. However, in the current study no significant differences in sarcomere lengths between the treatments were observed.

Shrinkage of meat during cooking can occur both transverse and longitudinal to the fibre direction, and shrinkage affects cooking loss. Longitudinal shrinkage may be a consequence of both sarcomere shortening and collagen shrinkage. Micklander *et al*. (2002) found a relationship between longitudinal shrinkage and T_2 relaxation times at 57°C in porcine *longissimus dorsi* and taken as longitudinal shrinkage in the present study, sarcomere length were measured on samples heat treated at 53°C and 59°C for 3 and 20 h, but no significant differences were observed between the treatments. However, Offer, Restall & Trinick (1984) and Palka & Daun (1999) observed that shrinkage of meat during cooking at 45-90°C occur in two phases; at 45-60°C the shrinkage is primarily transverse and at 60-90°C primarily longitudinal to the fibre direction. According to this, transverse and not longitudinal shrinkage may have occurred during LTLT treatment in the present study, thus, degree or direction of shrinkage may have caused the division of NMR T_2 relaxation times according to temperatures in Figure 1.

The influence of LTLT treatment on shear force is illustrated in Figure 3.

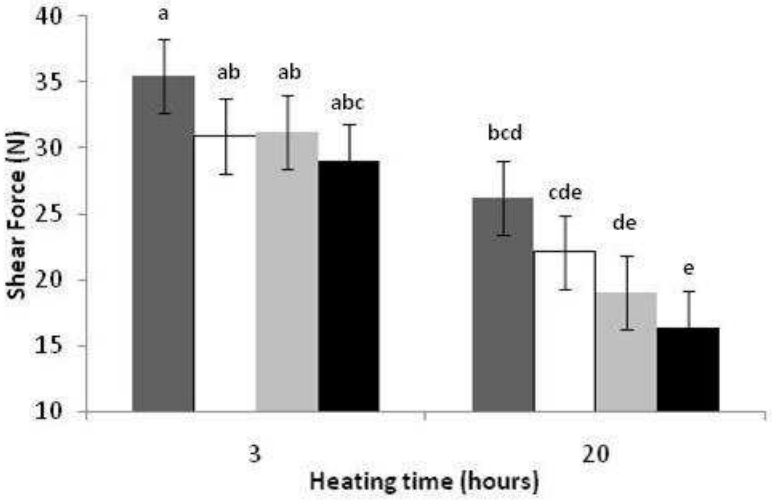

Figure 3. Effect of time and temperature of Warner-Bratzler Shear Force (N) of LTLT treated porcine *Longissimus dorsi*. Dark grey: 53°C; white: 55°C; light grey: 57°C; black: 59°C. Bars represent standard errors (n=3).

Increasing cooking time from 3 to 20 hours decreased shear force significantly ($P<0.01$) at all 4 temperatures. At 20 hours of cooking shear force was significantly lower at 59°C compared to 53°C ($P=0.03$), while no significant differences in shear force were found after 3 hours of cooking. An inverse relationship between cooking loss (Figure 2) and shear force (Figure 3) in LTLT treatment was observed, since, when shear force decreased, cooking loss increased.

The decreased toughness at 20 hours of cooking in the present study could be ascribed to other factors as e.g. proteolysis or solubilisation of collagen.

IV. CONCLUSION

Low temperature long time treatment of porcine *longissimus dorsi* between 53°C and 59°C revealed decreased toughness and increasing cooking loss with increasing temperature. Furthermore, by increasing heating time from 3 to 20 h, shear force decreased and cooking losses increased. NMR T_2 relaxation times revealed that changes occurred between 55°C and 57°C, however, further investigations are required to explain these changes.

ACKNOWLEDGEMENT

The authors would like to thank Peder Vithus Johansen (DMRI) for helping with the collection of samples, and Nina Eggers (Aarhus University) for technical assistance in the NMR laboratory. The authors thank The Danish Ministry of Food, Agriculture and Fisheries for funding the projects entitled "Safety and gastronomic quality of new LTLT treatments of meat" and "Integrated characterization of food quality and microbial safety".

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