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Effect of prolonged heat treatment from 48°C to 63°C on toughness, cooking loss and color of pork

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ABSTRACT

The effect of low temperature long time (LTLT) heat treatment at 48 °C, 53 °C, 58 °C, and 63 °C for T_c (time to reach a core temperature equal to the water bath), $T_c + 5$ h holding time, and $T_c + 17$ h holding time was studied in *Longissimus dorsi* and *Semitendinosus* muscles from slaughter pigs and sows. Meat toughness (Warner–Bratzler Shear Force), cooking loss and color (Minolta L*a*b*-values) was measured and in the cooking loss the amount of heat-soluble collagen and activity of cathepsin B + L was determined. Decreasing shear force and increasing cooking loss during LTLT treatment was observed between 53 °C and 58 °C. Furthermore, increasing temperature from 53 °C to 58 °C and increasing time from T_c to $T_c + 17$ h increased the solubility of collagen. Residual activity of cathepsin B + L in LTLT treated pork was mainly affected by temperature, showing the highest activity at 58 °C and 63 °C.

1. Introduction

Tenderness of meat is one of the main quality criteria to consider in the determination of the most desirable cooking conditions. Davey and Gilbert (1974) defined cooking of meat as "heating to a sufficiently high temperature to denature proteins". Changes in meat tenderness which occur during cooking are generally associated with heat-induced alterations of the components of the primary structure of the muscle tissue such as collagenous and myofibrillar proteins. Studies have shown that meat is more tender and has less cooking loss when heating temperatures are low (Bramblett, Hostetler, Vail, & Draudt, 1959; Penfield & Meyer, 1975; Bejerholm & Aaslyng, 2004). Several studies (Bramblett et al., 1959; Machlik & Draudt, 1963; Tuomy, Lechnir, & Miller, 1963; Bramblett & Vail, 1964; Laakkonen, Wellington, & Sherbon, 1970; Penfield & Meyer, 1975; Beilken, Bouton, & Harris, 1986; Christensen, Purslow, & Larsen, 2000) have found a pronounced decrease in shear force in beef muscles on heating from 50 °C to 60 °C.

Clearly temperature, but also duration of cooking has a large effect on the physical properties of meat and the eating quality. But the effect on these physical properties when meat is heated at lower temperatures for a long period of time (LTLT) is not clearly understood, although Bramblett et al. (1959) and Bramblett and Vail (1964) concluded that the length of holding time between 57 °C and 60 °C was closely related to tenderness in a range of beef muscles.

Davey and Niederer (1977) suggested that tenderization of meat by cooking at temperatures up to 100 °C, takes place by specific proteolytic attack on the myofibrillar structure at temperatures up to 65 °C, and by destruction or weakening of collagen at temperatures above 70 °C. This interpretation, however, does not agree with a range of studies on cooking of meat at low temperatures (Laakkonen, Sherbon, & Wellington, 1970; Penfield & Meyer, 1975; Bouton & Harris, 1981; Beilken et al., 1986; Christensen et al., 2000) which suggested a weakening of collagen well below 70 °C. Christensen et al. (2000) found that the decrease in whole meat toughness upon cooking at temperatures between 50 °C and 60 °C was associated with a decrease in the strength of the perimysial connective tissue, whereas the strength of the muscle fibers increased above 60 °C. Beilken et al. (1986) found that at 55 °C and 60 °C the connective tissue strength of bovine *Semimembranosus* decreased significantly when increasing heating time up to 4 h in 2–3 month old calves and up 48 h in 17 year old steers. Moreover, Bouton and Harris (1981) reported decreased connective tissue strength, measured by adhesion, when the temperature was increased from 50 °C to 60 °C or when heating time was increased from 1 to 24 h. Accordingly, by investigating changes in porcine epimysium between 50 °C and 60 °C, Bruggemann, Brewer, Risbo, and Bagatolli (2010) showed, using Second Harmonic Generation microscopy, that at 57 °C collagen shrinkage occurred and at 59 °C the signal corresponding to collagen vanished. Increased tenderness of LTLT treated meat may also be attributable partially to the action of proteolytic enzymes, causing weakening of myofibrils,

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and weakening and/or solubilization of collagen. Laakkonen, Sherbon, et al. (1970), Laakkonen, Wellington, et al. (1970) and Penfield and Meyer (1975) found that activity of collagenase and other proteolytic enzymes could still exist for prolonged times at temperatures below 60 °C, while at higher temperatures these enzymes were inactivated. Therefore the potential causes for tenderization due to LTLT treatment are: (a) weakening of structural proteins in myofibrils and (b) increased solubilization of collagen.

Even though comprehensive investigations have been made on cooking of meat, no studies have investigated the effect of LTLT treatments between 48 °C and 63 °C on porcine muscles. The objective of the current study was to investigate the effect of LTLT treatment on toughness, cooking loss and color of *Longissimus dorsi* and *Semitendinosus* muscles from both slaughter pigs and sows. Furthermore, solubility of collagen and residual activity of cathepsin B + L as affected by LTLT treatment were investigated.

2. Materials and methods

2.1. Raw materials

Longissimus dorsi (LD) were obtained from both sides of 12 slaughter pigs and 12 sows. Seventy two *Semitendinosus* (ST) were obtained from other slaughter pigs and 21 ST from other sows. pH values (measured 24 h *post mortem*) and slaughter weights are listed in Table 1. Whole muscles (LD and ST) were vacuum packed and stored at 4 °C for 4 days. After storage muscles were cut into samples (according to the design), weighed, vacuum packed and frozen at −20 °C.

2.2. Design

LD from slaughter pigs and sows was cut into 3 samples each about 5 × 12 × 11 cm (height × length × width). ST from sows was cut into 2 samples about 13 cm in length, and whole ST from slaughter pigs equated to one sample about 13 cm in length. Twelve LTLT treatments were assigned to samples from different animals and different locations on the muscle in an incomplete block design. An exception was ST from sows which were only given 7 of the 12 LTLT treatments. Each LTLT treatment was repeated 6 times.

2.3. LTLT treatment

Frozen samples were thawed overnight at 4 °C and LTLT treated in the vacuum bag in water baths (Roner, ICC, Frinox, Hillerød, Denmark) at 48 °C, 53 °C, 58 °C, and 63 °C (±0.05 °C). Heating times were T_c (time to reach a core temperature equal to the water bath) (LD: 3 h; ST: 2 h), T_c + 5 h holding time and T_c + 17 h holding time. LTLT treatments were arrested by keeping the samples in iced water for 10 min. After LTLT treatments the meat were weighed and the cooking loss was calculated as the weight lost during LTLT treatments. Cooking loss was collected, centrifuged at 5072 × *g* for 15 min at 4 °C and subsequently frozen at −80 °C. LTLT treated meat samples were stored in vacuum bags overnight at 4 °C and then analyzed for Warner–Bratzler Shear Force and instrumental color.

Table 1
Means of slaughter weight, pH_{24}, total collagen and heat soluble collagen of *Longissimus dorsi* (LD) and *Semitendinosus* (ST) from slaughter pigs and sows. Standard deviations are shown in parentheses.

		Pigs	*n*	Sows	*n*
Slaughter weight (kg)		79 (±2)	12	172 (±40)	12
pH24	LD	5.62 (±0.05)	24	5.56 (±0.07)	24
	ST	5.94 (±0.19)	72	5.84 (±0.23)	21
Total collagen (mg/g meat)	LD	4.2	[a]	5.3 (±0.7)	12
	ST	7.7	[a]	8.0	[a]
Heat soluble collagen	LD	34.4%	[a]	12.5% (±3.4%)	12
(% of total collagen)	ST	39.5%	[a]	16.5%	[a]

[a] All raw materials within each muscle and type of animal were pooled.

2.4. Warner–Bratzler Shear Force

Meat samples were cut into 6 blocks of 1 × 1 × 6 cm. Each block was sheared 3 times by TA-HDi Texture Analyzer (Stable Micro Systems, UK) equipped with a triangular Warner–Bratzler test cell. Mean maximum force required to shear through the samples, Warner–Bratzler Shear Force, was determined from each LTLT treatment.

2.5. Color

A slice about 2–3 cm in length was cut from the hip end of the LTLT treated samples and the color (L*, a* and b* values) of the meat was measured 4 times on the fresh cut surface of the large part of the sample with a Minolta CR-300 Chroma meter (Minolta Camera Co., Osaka, Japan). Color of LTLT treated ST was measured on the 'white' part of the muscle. Mean values from six repetitions on each LTLT treatment were obtained.

2.6. Cathepsin B + L activity

Residual activity of Cathepsins B and L in the fluid lost on cooking (cooking loss) was measured fluorometrically. The cooking loss was thawed at room temperature and 15 µl mixed with 135 µl of buffer (340 mM sodium acetate, 60 mM 100% acetic acid, 4 mM EDTA, 0.1% Brij 35, pH 5.5) and heated to 40 °C. Samples were incubated with 100 µl substrate (12.5 µM Z-Phe-Arg-Nmec (Sigma)) for 10 min at 40 °C. The reaction was arrested by adding 1 ml stop buffer (100 mM NaOH, 30 mM sodium acetate, 70 mM 100% acetic acid, 100 mM chloroacetic acid, pH 4.3). To each well in a 96 well microtiter plate 250 µl sample was added, and measured at excitation and emission wavelengths of 355 nm and 460 nm, respectively. Standards were made by 7-amino-4-methyl coumarin (Sigma) and stop buffer was used as the blank. The activity was expressed in µU/g meat where 1 unit (U) was defined as 1 µmol product produced per minute at 40 °C.

2.7. Heat soluble collagen in the cooking loss

The concentration of heat soluble collagen was measured only in samples LTLT treated at 53 °C and 58 °C. Cooking loss was thawed at room temperature and 500 µl, transferred to a Cryotube with cap and mixed with 500 µl 6 M HCl (in duplicate). Tubes were placed in a sand bath for hydrolysis at 115 °C for about 20 h. Hydrolysates were then diluted to 5 ml with water and frozen at −20 °C until measurement of hydroxyproline concentration. Measurement of hydroxyproline concentration was conducted according to Kolar (1990) with slight modifications. Absorbance was measured in 200 µl at 560 nm. Concentration of heat soluble collagen in the cooking loss was expressed as mg soluble collagen/g meat.

2.8. Total- and heat soluble collagen

Total- and heat soluble collagen content was determined from raw LD and ST from slaughter pigs and sows before LTLT treatment as described by Kristensen et al. (2002). Due to large variations in slaughter weight of the sows used (Table 1), total- and heat soluble collagen in LD from sows were measured on each animal. Total- and heat soluble collagen in LD from slaughter pigs and ST from both slaughter pigs and sows were measured on homogenates containing raw meat from all animals included in the investigation of the specific muscle.