TENDERIZATION OF BEEF BY LACTIC ACID TREATMENT APPLIED AT DIFFERENT TIMES POST-MORTEM (*)

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BACKGROUND AND OBJECTIVE

Treatments consisting in applying acid solutions have long been used as a means of reducing the background toughness (or connective tissue toughness) and consequently, to improve the overall tenderness of collagen-rich beef muscles prior to cooking. Marination in lactic or acetic acid solutions decreases the mechanical resistance of meats (Wenham and Locker, 1976; Lewis and Purslow, 1991; Kijowski and Mast, 1993; Ertbjerg et al., 1995). However, the slow penetration of exogenous acids in the meat limits the efficiency of this treatment (Seuss and Martin, 1993; Kotula and Thelappurath, 1994). Therefore the injection of the acid solution allows a much more rapid diffusion in the muscle mass and results in a lower mechanical strength and a higher tenderness rating (Cannon et al., 1993; Eilers et al, 1994; Ertbjerg et al, 1995). The tenderizing effect of acid treatments was first related to an increase in collagen solubility (Stanton and Light, 1990; Oreskovich et al., 1992; Ertbjerg et al, 1995). Studies on pre-rigor beef muscles showed that acid treatments also accelerate the release of lysosomal proteases (Ertbjerg et al; 1994, 1995), thus possibly causing the post-mortem solubilization of collagen (Stanton and Light, 1990; Ertbjerg et al., 1995). The objective of this experiment was to investigate the tenderizing potential of the injection of lactic acid in collagen-rich beef muscles according to the time post-mortem (pm) of injection and the duration of the storage period.

MATERIALS AND METHODS

Eight Friesian cull cows (age 3 to 4 years) were slaughtered at 256 (± 39) kg carcass weight. The *M. Pectoralis profundus* was excised from both sides of each carcass immediately after slaughter. A square sample (approx. 25 × 25 cm) was cut from the central part of the muscle of each side, and then divided into 6 subsamples of similar size (approx. 7 × 10 cm). The 12 subsamples obtained from each animal were allocated randomly to 6 treatments according to a 3 × 2 factorial design. The treatments were the combinations of 3 modes of application of lactic acid (control– C, lactic acid injected either at 1 h– LA-1, or at 24 h pm– LA-24) with 2 storage durations (2 or 14 days pm). The treated samples were injected with a 0.5 M lactic acid solution (10 % w/w) with approximately 0.5 cm between injection points, using a multi-pipette equipped with an adaptor with fixed needles. The controls were only punctured. All samples were vacuum packed at 4 h pm and kept until 24 h pm at 15°C. After the post-rigor injection (LA-24) was performed, all samples were stored at 4°C until completion of their respective storage period, and subsequently frozen at -20°C until analysis. Subcellular fractionation was carried out by differential centrifugation on muscle homogenates. Fluorimetry was used to measure the combined activities of cathepsins B and L (Kirschke et al., 1983) and the β-glucuronidase activity (Moeller et al., 1976) in the membrane (mitochondrial + lysosomal + microsomal) and soluble fractions. Myofibrillar protein degradation was followed by SDS-PAGE. The total collagen and the insoluble (90 °C, 2 h) collagen contents in the raw meat were determined. Samples of isolated perimysium were used to determine the transition temperature of collagen by differential scanning calorimetry (DSC; heating rate 3 °C/min). Tension was measured isometrically while heating from 15 to 97 °C (rate 3 °C/min) a muscle sample (40 × 5 × 5 mm) restrained at its resting length in an isotonic buffer solution. Then, the same sample was used to determine the tensile strength of the cooked meat using an Instron universal testing-machine (displacement rate 50 mm/min). The initial yield (myofibrillar toughness, M-force) and final yield (connective tissue toughness, C-force) were determined from the deformation curves (Warner-Bratzler shear test using triangular blade on Instron machine) obtained on meat cubes (1 × 1 × 5 cm) after cooking for 120 min at 60 °C (Møller, 1981). Sensory analysis was performed on cooked (120 min at 60 °C) meat from 4 cows. Tenderness was evaluated (ease of first bite, chewiness) by trained panelists using a 10-point scale (1 = very tough, 10 = very tender).

RESULTS

In the LA-1 treatment, the pH value dropped to 5.0 within 4 h pm and it stabilized at 5.0 at 24 h pm (ultimate value, pHu). At this time, pH was 5.6 in the control (pHu). LA-24 injection resulted in a further decline to a pHu value of 4.9 measured at 28 and 48 h postmortem. Both at days 2 and 14 pm, LA-1 and LA-24 increased to the same extent (+126%; $P < 0.001$) the cathepsin B+L activity in the soluble fraction. Concomitantly, the activity was reduced in the membrane fraction by 30% ($P < 0.001$). Also β-glucuronidase activity increased ($P < 0.01$) in the soluble and decreased ($P < 0.001$) in the membrane fraction. Electrophoresis showed that the major effect of LA injections on the degradation of myofibrillar proteins was a marked decrease in the density of the band of the myosin heavy chain (MHC) and the appearence of a 150 kDa band, with no effect of injection time. This effect was large at 2 days pm, and it was still perceptible after 14 days. Actin degradation was small, and the 31-kDa band (troponin T) was only affected by storage duration.

Lactic acid injections reduced ($P < 0.001$) the meat insoluble collagen content, with no effect of storage duration. The decrease averaged 6% and 10 % in LA-1 and LA-24 treatments respectively, corresponding to values of collagen heat solubility of 16 and 18% against 11% in the controls. The isometric tension curves showed that the heat-induced contraction of the muscle tissue started at a lower temperature in injected samples (-1.2 and -2.6 °C temperature difference for LA-1 and LA-24 respectively compared with controls). They also showed 2 transitions at around 65 and 75°C. The first one could be assigned to the denaturation of myosin and collagen, and the second one to actin. In injected samples, the transition temperatures were 1.2 to 2.6 °C lower ($P < 0.001$), and stress at the second transition was 12 to 28 % lower ($P < 0.001$). The decrease in the transition temperature of collagen was confirmed by the DSC measurements on isolated perimysium. The onset temperature was 0.6 and 1.5 °C lower in LA-1 and LA-24 treatments respectively ($P < 0.001$).

Tensile strength of cooked meat was lower by 13% (LA-1; $P < 0.01$) to 20% (LA-24; $P < 0.001$) in injected samples stored for 14 days. Correspondingly, LA injection reduced ($P < 0.001$) the shear force components. After a 2-day storage period, the reduction was 36% (M-force) and 15% (C-force), with no effect of injection time. Further ageing to 14 days reduced M-force ($P < 0.05$), but only in C samples.

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After 14 days, M-force in C samples was still higher than that in injected samples. Storage duration did not change C-force in any treatment. Texture sensory scores were also improved by lactic acid injection ($P < 0.05$), the treatment being equally efficient at both injection times. The injections, particularly LA-1, caused dramatic, but heterogeneous, changes in the myofibrillar ultrastructure, such as the coagulation of the myofibrillar components, the weakening or the rupture of I-bands and a marked weakening of M-bands. These effects were observed in some LA-1 samples and in all LA-24 samples, both at days 2 and 14 pm. Some parts of the muscle sections exhibited a normal appearance, particularly in LA-1 samples, showing that they had probably not been affected uniformly by the treatment. As a general rule, the extent of the structural alterations caused by lactic acid injection was greater in the LA-24 treatment.

DISCUSSION

The results of this experiment provide clear evidence that, independently of the time post-mortem of injection (pre- vs. post-rigor), a 0.5 M lactic acid injection was efficient in reducing the toughness of a collagen-rich beef muscle such as *M. Pectoralis profundus*. Previous works have already demonstrated the positive effect of acidic treatments on beef texture, the acid being applied either by marination (dipping) (Wenham and Locker, 1976; Lewis and Purslow, 1991; Rao and Gault, 1990) or by injection (Eilers et al., 1994, Ertbjerg et al., 1995). In some works, marination was reported to have no beneficial effect on beef tenderness (Seuss and Martin, 1993; Kotula and Thelappurath, 1994), but this could be attributed to the limited penetration of the acid into the muscle. The efficiency of an acidic treatment also depends upon the intrinsic toughness of the meat (Wenham and Locker, 1976) and the acid concentration (Rao and Gault, 1990; Oreskovich et al., 1992). Ertbjerg et al. (1995) showed that lactic acid injected at low concentrations (0.3 M) does not improve beef texture because it leads to a pH value close to the isoelectric point of the major myofibrillar proteins, while at higher concentrations (1.0 M) it decreases meat toughness.
Obviously, the improvement of meat texture achieved in the present work was the result of the weakening of both the connective tissue and the myofibrillar structure, as suggested previously by Rao and Gault (1990). The DSC measurements showed that the perimysial collagen underwent changes at the molecular levels under acidic conditions, which confirms the findings of Kijowski (1993). The lower denaturation temperatures of collagen are consistent with the increased heat solubility of this protein, which was also reported by Oreskovich et al. (1992) and Ertbjerg et al. (1995). The swelling of the perimysial collagen caused by the acidification of the medium (Kijowski and Mast, 1993) may also be responsible for the lower strength of the raw perimysium (Lewis and Purslow, 1991) and the higher propensity of this tissue to heat denaturation and shear deformation. The weakening of the M-bands and the coagulated aspect found in the myofibres of injected samples confirms the observations made by Rao et al. (1989) and Oreskovich et al. (1992) at meat pH values below 4.5 with acetic acid.
The present results show that lactic acid injection tended to be more efficient when performed post-rigor instead of pre-rigor, in particular on the collagen fraction. However, this difference was not significantly reflected in the overall toughness of the cooked meat (shear value, sensory scores). Actually, pre- and post-rigor injections accelerated the release of the lysosomal enzymes into the cytosol, thus confirming the works of Ertbjerg et al. (1994 and 1995). But the amplitude of this effect was the same at day 2 pm in both treatments. The much faster rate of pH fall pm in pre-rigor injected samples may have limited the proteolytic activity of calpains. This could explain the greater extent of the post-mortem degradation of the myofibrillar ultrastucture in the post-rigor injected meat.

CONCLUSION

The injection of 0.5 M lactic acid reduced markedly the toughness of a collagen-rich beef muscle (*M. Pectoralis profundus*) as soon as 2 days post-mortem. Both the background toughness (connective tissue) and the myofibrillar toughness were favourably affected. The time post-mortem of injection (pre- vs. post-rigor) had only a minor influence on the short- and long-term efficiency of the treatment. However, further investigation is needed to determine the extent of the detrimental effects of this treatment on meat colour and flavour that could limit the consumer acceptability of the injected meat.

Table 1. Effect of lactic acid injection on the insoluble collagen content, texture and enzyme activity of beef according to time post-mortem.

Treatment	Insol. collagen content (mg/g)		Tensile strength (N/g DM/cm)	WB-M force (N)		WB-C force (N)		Ease of first bite (*)		Cathepsin B+L activity (mU/g muscle)			
										Soluble fraction		Membrane fract.	
	Day 2	Day 14	Day 14	Day 2	Day 14	Day 2	Day 14	Day 2	Day 14	Day 2	Day 14	Day 2	Day 14
Control	10.3a	10.5a	6.9a	40.1a	30.0a	43.9a	43.8a	4.36a	4.22a	0.74a	1.17a	0.90a	0.88a
LA-1	9.5b	10.1a	6.0b	25.9b	25.4ab	28.8b	33.3b	6.83b	6.00b	1.67b	2.05b	0.64b	0.61b
LA-24	9.0c	9.6b	5.5c	25.6b	24.8b	27.2b	26.9b	7.49b	6.39b	1.67b	2.22b	0.52b	0.62b

(a,b,c) means in the same column accompanied by different letters are significantly different ($P < 0.05$).
(*) sensory panel score using a scale from 1 (very tough) to 10 (very tender).

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