E64

LACTIC ACID TREATMENT FOR UPGRADING LOW QUALITY BEEF

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Background and objectives

Improved tenderness in poorer quality muscles with a high collagen content would be appreciated by the meat industry. A strategy is to degrade the intrinsic collagen structure to produce the desired tenderness and texture. Pre rigor injection of beef with 0.1 M lactic acid resulted in increased solubilisation and degradation of perimysial collagen (Stanton and Light, 1990). Improved ternderness after injection of 0.3 M lactic acid was reported (Eilers *et al.* 1994). Pre rigor injection of hot boned beef with 0.3 M lactic acid resulted in a rapid and enhanced release of lysosomal enzymes (Ertbjerg *et al.* 1994). Cleavage of native collagen by the lysosomal enzymes cathepsin B and L has been shown (Burleigh *et al.* 1974; Kirschke *et al.* 1982; Mason *et al.* 1984). Also myofibrillar proteins can be degraded by cathepsin B and L (Ouali *et al.* 1987; Mikami *et al.* 1987).

The aim of this work was to investigate the potential for upgrading lower quality beef by pre rigor lactic acid treatment. The effect on meat texture of pre rigor 0.3 M or 1.0 M lactic acid injection was investigated in hot boned beef. The ultimate pH were decreased below the isoelectric point of the major myofibrillar proteins by injecting 1.0 M lactic acid. As an attempt to evaluate if effects were due to enzymes, an inhibitor of cysteine proteinases (E-64) was used and electrophoresis of myofibrillar proteins performed.

Methods

Animals and muscle: All experiments were performed on *M. Pectoralis Profundus* (PP) removed from the carcasses of 3 to 4 year old cows within 60 min post slaughter. A square (approximately 25 x 25 cm) in the middle part of the muscle was used cutting away the triangeled areas at the anterior and posterior ends and thinner areas at the dorsal and ventral sides. The square was furthermore divided into subsamples of equal size before injection of lactic acid.

Lactic acid injection: Lactic acid was injected using a multi pipette with fixed needles. Subsamples of the PP were injected with lactic acid to a level of 10 % of the original weight of the muscle. Injections were performed in three depths with approx. 0.5 cm between each needle injection point. After 24 hr at 15 °C, the samples were vacuum packed before conditioning at 15 °C to 3 days postmortem.

Shear force measurements: Measurements were performed essential as described (Møller, 1981). Briefly, an Instron Universal testing machine was used. On each of four meat blocks, three shear force deformation curves were obtained. From the WB deformation curves two parameters were measured. (1) WB M-force or initial yield taken as a measurement of the myofibrillar component of tenderness. (2) WB C-force or final yield taken as a measurement of the connective tissue component of toughness.

Total and heat soluble collagen: Meat samples (6 g) were minced . After addition of 20 ml 0.9 % NaCl heat treatment was performed at 90 °C for 120 min. After centrifugation (4,000 *g* for 15 min) the residue was rewashed with 20 ml 0.9 % NaCl at 40 °C and centrifuged again. The two supernatants were combined and made up to a volume of 50 ml. The residue and the combined supernatant were analysed for hydroxyproline content, and per cent of soluble collagen was calculated as outlined (Wyler, 1972).

SDS-PAGE: Electrophoresis of isolated myofibrils were performed on a 8 - 16 % gradient gel. Protein bands were quantified using a scanner.

Results and discussion

The effect of 0.3 M lactic acid injection as compared to control (no injection) was investigated. Muscles from 2 animals (left and right sides) were used. Tenderness was evaluated using two different heat treatments for the Warner-Bratzler analysis (60 °C and 70 °C for 60 min). Sample location 2 showed pronounced higher C-force values (Table 1), indicating that the PP muscle consists of a tough and a more tender part, mainly due to differences in the connective tissue content. Lactic acid injection resulted in slightly increased WB M-force values (initial yield) at both temperatures and both sample locations. Heat treatment of 60 °C resulted in a 8 - 16 % decrease in C-force values. In contrary, after heat treatment of 70 °C the C-force values increased 18 - 19% as a result of lactic acid injection. The overall effect of 0.3 M lactic acid injection thus seems neutral at low cooking temperatures (60 °C), lowering the connective tissue component and increasing the myofibrillar component of meat toughness. At higher cooking temperatures (70 °C) the effect of 0.3 M lactic acid was negative, thus increasing both WB-M and WB-C force. This is contradictory to the study (Eilers *et al.* 1994), showing lower shear force values as a result of 0.3 M lactic acid injection.

Table 1. Effect of lactic acid treatment on Warner-Bratzler shear force at two sample locations in Pectoralis Profundus muscle. Each result represents mean value of 4 samples (2 from each animal) and are shown in N/cm^2 ± standard deviation.

Lactic acid treatm.	Sample location[1]	WB M-force		WB C-force	
		60 °C	70 °C	60 °C	70 °C
Control	1	32.0 ± 4.1	42.2 ± 2.2	50.2 ± 4.5	37.4 ± 4.3
	2	30.8 ± 3.6	38.6 ± 1.9	79.6 ± 7.9	52.2 ± 7.0
0.3 M	1	34.8 ± 4.1	43.6 ± 6.6	42.1 ± 5.8	44.4 ± 6.5
	2	37.5 ± 3.5	48.2 ± 5.6	73.2 ± 19.6	61.6 ± 8.4

[1]Sample location 1: anterior part; sample location 2: posterior part of the muscle

Table 2. Effect of injected lactic acid (0.3 or 1.0 M) and E-64 on Warner-Bratzler M- and C-force, heat soluble collagen and cooking loss.

Lactic acid treatment	WB-M (N/cm^2) 60 °C	WB-M (N/cm^2) 80 °C	WB-C (N/cm^2) 60 °C	WB-C (N/cm^2) 80 °C	Cooking loss (%) 60 °C	Cooking loss (%) 80 °C	Heat sol. collagen (%)
0.3 M + E-64	50.2^{a}	66.7^{a}	62.7^{a}	70.5^{a}	36.8^{a}	48.0	16.1^{a}
0.3 M	35.8^{b}	50.5^{b}	$51.5^{a,b}$	$52.1^{a,b}$	$33.3^{a,b}$	44.7	17.7^{a}
1.0 M + E-64	21.3^{c}	30.5^{c}	$37.8^{b,c}$	$33.7^{b,c}$	28.2^{b}	40.8	24.6^{b}
1.0 M	16.4^{c}	22.2^{c}	28.8^{c}	25.6^{c}	29.6^{b}	40.5	24.9^{b}

^{a,b,c} Means (n = 4) within the same column with different superscripts differ ($P < 0.05$).

The effect of injecting 1 M lactic acid compared to 0.3 M lactic acid with or without 20 μM E-64 (an inhibitor of cysteine proteinases) was investigated using muscles from 2 animals (Table 2). Increasing the lactic acid concentration from 0.3 M (ultimate pH 5.2) to 1.0 M (ultimate pH 4.6) resulted in a pronounced reduction in WB M- and C-force values ($P < 0.01$), increased percentages of heat soluble collagen ($P < 0.01$) and reduced cooking loss at 60 °C ($P < 0.05$) but not significantly at 80 °C. The inhibitor resulted in higher shear force values and slightly reduced percentages of heat soluble collagen.

Myosin heavy chain (MHC, 200 kDa) was found to be more than 50 % degraded as a result of 1 M lactic acid injection (Fig. 1). A degradation product of MHC emerged as 140-165 kDa band(s). Degradations as a result of 1.0 M lactic acid injection were also pronounced in the following bands: 44 (actin), 38, 35 and 21 kDa. Increased intensities were especially seen at 27, 19 and 16 kDa bands. The degraded 21 kDa band seemed to emerge at 19 kDa. Scanning showed that the inhibitor tended to counteract the effects of lactic acid.

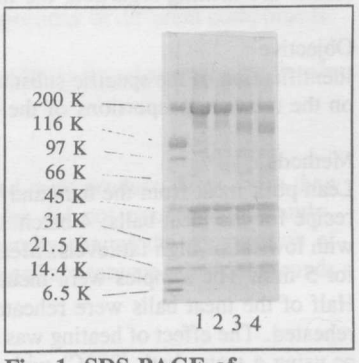

Fig. 1. SDS-PAGE of myofibrillar proteins.
lane 1: 0.3 M lactic acid + E-64;
lane 2: 0.3 M lactic acid;
lane 3: 1.0 M lactic acid + E-64;
lane 4: 1.0 M lactic acid.

In acid marination, decreasing the pH from pH 5.4 resulted in maximum toughness around pH 5.0 and increasing tenderness for lower pH values (Gault, 1985). The minimum water holding capacity of beef muscle homogenate is around pH 5.0 (Hamm, 1960), which corresponds to the mean isoelectric point of the major myofibrillar proteins. Therefore, the increased toughness of the myofibrillar component after injection of 0.3 M lactic acid could result from a decrease of the ultimate pH towards the isoelectric point of the major myofibrillar proteins resulting in decreased water holding capacity and increased toughness of the myofibrils. The positive effect on the myofibrillar component of toughness after 1.0 M lactic acid injection could have resulted from a decrease of the ultimate pH below the isoelectric point of the major myofibrillar proteins i.e. pH values below 4.7. Additionally, the results could be explained by increased activity of lysosomal enzymes. These enzymes are released from the lysosomes after lactic acid injection (Ertbjerg *et al.* 1994). Some of the changes observed as a result of lactic acid injection were caused by lysosomal enzymes, because the enzyme inhibitor E-64 counteracted the effects of lactic acid seen on shear force values and on the degradation pattern of myofibrillar proteins.

Conclusions

Lactic acid injection (0.3 M) generally resulted in increased shear force values for the myofibrillar component of meat toughness. The effect of 0.3 M lactic acid injection on the connective tissue component of meat toughness depended on the used cooking temperature: after heat treatment at 60 °C the C-force values decreased and after heat treatment at 70 °C the C-force values increased. Injection of 1.0 M lactic acid resulted in lower shear force values, higher percentages of heat soluble collagen and increased degradation of myofibrillar proteins as compared to 0.3 M lactic acid. Fresh meat colour was severely affected by 1.0 M lactic acid. The enzyme inhibitor E-64 counteracted the effects of increasing lactic acid concentration, suggesting the involvement of lysosomal enzymes in the mechanism of increased tenderisation by lactic acid.

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