Biochemical and quality characterites of ovine muscle as affected by electrical stimulation and mode of chilling

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Introduction

High temperature conditioning of the carcass as a method of preventing or reducing cold shortening and the accompanying toughness of meat has received considerable interest. It is generally accepted that cold shortening will cause muscle toughness when lamb (March and Leet, 1966; Marsh et al., 1968; McCrae et al., 1971) and beef (Locker and Hagyard, 1963) carcasses are chilled or frozen in the pre-rigor state. One approach used to prevent muscle toughening is to hold the carcass at 14 to 20ºC until pre-rigor changes in the muscle are near completion since minimum shortening occurs at this temperature range (Locker and Hagyard, 1963). In the case of lamb, at least a 16 hour holding period is required (McCrae et al., 1971). Cold shortening can be minimized by delaying the exposure of the carcass to cold temperatures until the muscle pH has reached a value below 6.0 and approximately 50% of the adenosine triphosphate (ATP) has been depleted (Bendall, 1975).

A carcass conditioning period may introduce an undesirable delay in processing. However, this problem can be resolved by electrical stimulation of the carcasses which ensures a fast drop in pH and a rapid depletion of muscle ATP (Carse, 1973; Locker et al., 1975; Bendall et al., 1976; Davey et al., 1976a and b; McCollum and Henrickson, 1977; Shaw and Walker, 1977; Savell et al., 1977; Bouton et al., 1978; Chrystall and Devine, 1978; Will et al., 1979; Elgasim et al., 1981; Whiting et al., 1981). Even though electrical stimulation has been adopted, little information is available regarding its combined effect with the mode of chilling. Recently, Rashid (1982) has found that the pH of the Longissimus dorsi (LD) and semitendinosus (ST) muscles reached nearly 6.0 afther 4 hours postmortem when lamb sides were electrically stimulated within 15 minutes after bleeding using direct current with square wave pulse at 350 voltage (V), 10 pulse per second (Hz) and 20 percent duty cycle (CD) when the sides were held at 16ºC. Some of the biochemical-biophysical changes wich take place may be related to meat quality. Hence, the aim of this study was to investigate the combined effect of electrical stimulation and slow chilling of lam carcasses at 16ºC for 5 hours postmortem on some physiochemical and quality characterisitics of specific ovine muscles.

Material and Methods

Animal and experimental desing.

Twelve Suffolk wether lambs (hot dressed carcass weight ranged from 21 to 29 kg) were slaughtered according to commercial practices (in the Abattoir of the Meat Science Laboratory, Oklahoma State University), skinned, eviscerated and divided into sides. The two sides within each carcass were randomly assigned to two different and a balanced incomplete block desing, block size 2, was used. Accordingly, a total of 12 sides were electrically stimulated (S) while 12 sides were kept as controls (C). In each case, 6 sides received rapid chilling treatment and the other 6 sides were subjected to slow chilling as shown in Table 1.

Electrical stimulation.

The sides electrically stimulated within 15 minutes post-mortem using a direct current with a square wave pulse for 4 minutes. Since a previous study (Rashid, 1982) had shown that electrical stimulation using 350 V with 10 Hz (20% DC) resulted in the highest rate of glycolysis as compared to some other combinations of different voltages and frequencies, these stimulation parameters were used in the present experiment. The electrical current was applied by two wires each terminated with a clamp. One clamp was attached to the neck region at the level of the 5th and 6th cervical vertebrae as the negative charge and the other clamp was attached to the achilles tendon (near its muscular attachment) as the positive charge to complete the circuit.

Muscle sampling procedure.

Two muscles, namely the LD and ST muscles were used to study the changes in some physiochemical and quality characteristcs. The ST muscle was excised from both the S and C sides inmediately after electrical stimulation. The extent of cold shortening, cooking loss and shear force value were determined at 24 hour post-mortem. Postmortem pH and temperature changes were monitored on intact LD muscles until 24 hours. Thereafter, samples were taken to measure the lean color, protein solubility, cooking loss and shear force value.

Muscle pH: Sample cores (1.27 cm in diameter) were taken drom intact LD muscles at 0, 2, 4, 6, 8 and 24 hours postmortem and 1.5 g samples (taken from the center of the cores) were homogenized with 15 ml of 0.005 M sodium iodoacetate (Nichols and Cross, 1980) for 30 seconds using a Brinkman Polystron. The pH of the slurry was measured with a Digital Corning-130 pH Meter.

Muscle temperature: The changes in the internal temperature of the LD muscles were measured with a temperature probe, (Koch Model 1364), at the same time intervals as for the pH measurements.

Muscle shortening: The ST muscle was divided longitudinally into 2 approximately equal weight and length strips. The initial lenght of each strip was marked by inserting common pins in either end. The strips were placed in deep trays, covered with Handi-W food wrap film (Dow Chemical Company, Midland, Michigan) to guard against evaporation and subjected as appropriate to either rapid or slow chilling as described in Table 1, then the final lenght of each strip was measured to calculate the percent shortening.

Cooking loss: The LD chops and ST strips were cooked to an internal temperature of 70ºC in a convection oven (Blodgett Co., Inc.). The heat penetration rate was monitored by a copper constantan thermocouple and a recording thermometer assembly (Honeywell Co., Electronik 15). Cooking losses were derived from the difference between weight of each chop or strip before and after cooking and expressed as a percentage of raw weight.

Shear force value: The cooked chops or strips were wrapped in a Handi W food wrap film and placed in a cooler at 2ºC for 12 hours to provide equalized firmness to insure uniform cores (Kastner and Henrickson, 1969). There cores (lateral, dorsal and medial) were taken from the LD muscles and two cores (1.27 cm in diameter) were obtained from the ST muscles parallel to the direction of fibers using a coring device with an d electrical drill. Two shear readings were recorded from each core at right angles to the muscle fiber using a Warner-Bratzler cell on the Instron Universal Testing Machine (Instron Corp., Model 1132). The drive and chart speeds were calibrated at 10 cm/min.

Lean color: Boneless loin chops were cut from the carcass 24 hours postmortem and allowed to bloom for 45 minutes. They were then placed on a styrofoam tray, wrapped in oxygen permeable commercial type film and placed in a retail case at 2ºC under 70 ft-candles fluorescent light for 4 days. Hunterlab L, a, b values which indicated respectively the lightness, redness and yellowness were measured at 24 hours intervals using Hunterlab Tristimulus Colorimeter, Model D25 L-9. The radio of redness to yellowness (a/b) was also calculated.

Protein solubility: The solubility of different protein fractions was performed according to the procedure of Asghar and Yeates (1974) with modifications.

Triplicate samples, 2 g each, from homogeneous minced LD muscle were extracted sequentially with different buffer systems. The sarcoplasmatic protein were extracted with 2% iso-osmotic glycerol solution (Scopes, 1970). The residue was extracted with 0.3 M NaCl unbuffer solution to dissolve myofibrillar protein and the with 0.6 M Kl in 0.1 M phosphate buffer to extrac the remaining myofibrillar proteins. All extractions and centrifugations were performed at 2ºC. The resulting residue after washing thoroughly with deionized water was extracted with chloroform-methanol (3:1, v/v) to remove lipid fractions. Thereafter, the residue was extracted with 0.1 M lactic acid to estimate the acid soluble collagen. Finally, the remaining residue again washed with deionized water, dried at 105ºC overnight, and designated as insoluble collagen fractions. The swelling factor was also estimated according to the procedure as described by Asghar and Yeates (1974). The protein content in different extracts was measured by biuret reaction and the A 540 nm was determined using a Gilford 240 Spectrophotometer (Gornall et al., 1949).

Statistical analysis.

The data were subjected to anlysis of variance using a balanced incomplete block desing, block size 2. The F-test was used to determine if significant differences occured among treatments. Means were compared by Duncan Multiple Range Test at the 5% level of significance (Steel and Torrie, 1960).

Results and Discussion

pH and temperature decline.

Both the electrical stimulation and the chilling methods had marked influence on muscle postmortem glycolisis (Figure 1). Stimulated sides whether rapid or slow chilled had a significantly (P 0.5) lower pH than the respective control muscles at 2, 4, 6, and 8 hours postmortem. This is in agreement with various researchers who have shown that electrical stimulation accelerates the rate of postmortem glycolisis (Carse, 1973; Bendall et al., 1976; Davey et al., 1976a; McCollum and Henrickson, 1977; Chrystall and Devine, 1978; Will et al., 1978; Whiting et al., 1981). However, muscle from electrically stimulated slow chilled sides (S + 16ºC) experienced a greater pH decline (P 0.5) than the rapid chilled (S + 2ºC) sides. On the other hand, postmortem pH fall in the controll sides whether slow or rapid chilled (C + 16ºC and C + 2º) was almost identical. Although it has been shown that the glycolitic rate increases with an increase in temperature above 10ºC (Marsh, 1954; Cassens and Newbold, 1966, 1967; Jeacocke, 1977), the difference in carcass chilling rates does not fully explain why electrically stimulated sides showed a more rapid pH fall in the case of slow chilling versus rapid chilling (Figure 1), since, in control sides, the pH fall was almost identical regardless of chilling temperature. It is possible that some glycolytic enzymes were activated during electrical stimulation to speed up glycolysis. This explanation is supported by Clarke el al. (1980) who found that phosphofructokinase, aldolase, glyceraldehyde 3-phosphate dehydrogenase and pyruvate bound to actin filaments in electrically stimulated muscles and increased the stability and activity of these enzymes. Whether or not electrical stimulation activates the enzyme system per se in muscle has not been completely defined.

The internal temperature of the LD muscle at 2, 4 and 6 hours postmortem of stimulated (S + 2ºC) and control (C + 2ºC) sides which were rapidly chilled was lower than the stimulated (S + 16ºC) and control (C + 16ºC) sides which were slow chilled (Figure 2). However, the temperature became almost similar for all treatments at 8 hours postmortem. There was no variation in temperature fall between the stimulated and control sides at any given chilling procedures (rapid or slow). Hence, differences in the rate of pH fall for the LD muscle between stimulated and control cannot be ascribed to difference in carcass temperature fall assumed by Bendall (1980). Activation of glycolytic enzymes by electrical stimulation may be one of the causative factors accounting for the rapid pH fall in electrically stimulated carcasses.

Muscle shortening

The effect of electrical stimulation and mode of chilling in reducing the cold shortening in the ST muscle is summarized in Table 2. Both the electrical stimulations and chilling treatments significantly influenced cold-shortening. The stimulated and slow chilled sides (S + 16ºC) had significantly less (P .05) shortening than the control groups. However, there was no significant difference (P .05) in the percent of shortening of the ST strips from electrically stimulated sides, whether they were rapid or slow chilled. On the other hand, ST strips from control sides shortened significantly more on rapid chilling. The present study shows that electrical stimulation significantly reduced muscle shortening which may be attributed to rapid depletion of the energy rich phosphate compounds (adenosine triphosphate and phosphocreatine) as they determine the response of muscle fiber shortening during chilling or freezing of carcasses (Asghar and Henrickson, 1982). Many researchers have reported that electrical stimulation accelerates musculature ATP depletion (Bowling et al., 1978; Will et al., 1980; Whiying et al., 1981). However, cold shortening is highly temperature dependent, being least severe between 15 and 18ºC (Harris, 1975). This study supports previous reports in that, by reducing the thime required for muscles to reach pH 6.0 (through the application of ES), and by holding carcasses for about less than 5 hours at 16ºC, the extent of cold shortening was reduced as compared to carcasses conventionally chilled at 2ºC.

Shear force and cooking loss.

The effect of electrical stimulation and holding temperature on the shear force value (kg) and cooking loss (%) for ST and LD muscles are also summarized in Table 2. Electrical stimulation significantly (P .05) decreased shear force value as compared to those from the control regardless of the postmortem chilling procedure for both ST and LD muscles. Most investigators have shown that electrical stimulations of the carcasses produced a tenderizing effect on the musculature (Carse, 1973; Chrystall and Hagyard, 1976; Davey et al., 1976b; Grusby et al., 1976; Ray et al., 1978; Stiffler et al., 1978; Cross 1979; Nilsson et al., 1979; Savell et al., 1979; Smith et al., 1979; Riley et al., 1980b; Bouton et al., 1980; Taylor and Marshall, 1980; McKeith et al., 1981). However, the shear force value of the ST muscle (excised from electrically stimulated sides) was significantly (P .05) less when it was slow chilled as compared to rapid chilled; whereas the electrically stimulated sides of intact LD muscle did not show significant differences in shear values between modes of chilling. Several explanations have been given by different researchers to account for improvements in tenderness from electrical stimulation. They include a) prevention of cold shortening (Bendall et al., 1976; Davey et al., 1976a; Gilbert et al.,1976; Walker et al., 1977; Bouton et al., 1980), b) increase in autolytic enzyme activity (Sorinmade et al., 1978; Dutson et al., 1980), and c) physical disruption of muscle fiber (Savell et al., 1978a; George et al., 1980).

With respect of the cooking loss, the data indicated no significant differences (P .05) in both ST and LD muscles as affected by electrical stimulation and chilling temperature (Table 2). This is in agreement with Riley et al. (1980b) and Thompson (1981). However, Savell et al. (1978b) noted a high cooking loss from electrically stimulated meat. These studies are not directly comparable as different stimulation techniques and conditions were used.

Lean color.

The lean color measurements using Hunterlab L, a , b values and the a/b color ratio of LD loin chops at 24 hours intervals for 4 days are shown in Figure 3. The treatment X day interaction exhibited no influence (P .05) on L, a, b color values and a/b color ratio. The data also indicated no significant differences (P .05) in the objective Hunterlab color values of meat among all treatments. This was contrary to the finfing of Riley et al., (1980a) who showed by subjective evaluation that electrical stimulation improved muscle color, decreased surface discoloration, and improved overall apperance of boneless loin chops from lambs during 4 days of display. Most of the studies, based on panel evaluation, have found the meat from stimulated carcasses generally to be brighter (Smith et al., 1977 1979; Savell et al., 1978a and b, 1979) and had a more youthful lean color (McKeith et al., 1981) than that from unstimulated carcasses. However, several workers agreed that electrical stimulation did not improve lean color (Grusby et al., 1976; Nichols and Cross, 1980).

Protein solubility.

The effect of electrical stimulation and chilling rate on the solubility of different protein fractions from the LD muscle, at 24 hour postmortem is shown in Table 3. Neither electrical stimulation nor the chilling rate had any significant effect (P .05) on the solubility of the saroplasmic protein fractions as compared to the control. This is in diagreement with George et al. (1980) who have concluded that slow cooling of electrically stimulated carcasses causes denaturation and precipitation of sarcoplasmic proteins onto the myofibrillars. If such a deposition occurs, it should reflected in decreased sarcoplasmis protein solubility. No change was unbuffered 0.3 M NaCl solution followed by 0.6 M Kl in 0.1 M phosphate buffer. It is generally thought that presence of the PO_4 ion in the buffer dissociates the actomyosin complex and hence probably increased the solubility of the myofibrillar protein (Mihalyi and Rowe, 1966). In view of this proposition, the myofibrillar proteins were first extracted with unbuffered 0.3 M NaCl solution to see whether or not actomyosin complex fomed to a different degree as a result of the different treatments applied to the carcass sides. It seems solubility test with unbuffered 0.3 M NaCl solution had no significant effect on the extent of actomyosin formation. Similary, the total percentage of myofibrillar protein and the intracellular protein were also not significantly different (P .05) among treatments. These observations agree with those of McKeith et al. (1980) who found no measurable differences in the solubility of the myofibrillar protein of muscle from electrically stimulated and unstimulated steer carcasses. Acid-soluble collagen (freshly synthesized collagen) and acid-insoluble collagen (biologically mature collagen and some elastin) fractions of the stroma protein were not significantly affected by electrical stimulation and carcass chilling (Table 3). The swelling factor which is used as an indicator of changes in the extent of crosslinkage of collagen (Asghar and Yeates, 1974, 1979) was also not affected by electrical stimulation. On the other hand, Judge et al. (1980) found no increase in the solubility of the perimysial collagem from electrically stimulated muscle. However, their data on differential scanning colorimetery showed a significant decrease (0.6ºC) in the thermal stability of the perimysial collagen of the L. dorsi muscle from electrically stimulated carcasses as compared to that from the control. As a matter of fact, very limited information is available on the influence, and more information is needed.

Conclusions

This study shown that the combined effect of electrical stimulation and mode of chilling profoundly affect some biochemical, biophysical and quality characteristics of ovine muscles. The sides which were electrically stimulated and slowly chilled (holding the carcass sides for 5 hours at 16ºC) exhibited more rapid pH decline, less cold shortening and greater tenderness than those which were subjected to other treatments. However, the lean color during a 4-day retail display and the solubility of different protein fractions showed no improvement by both electrical stimulation and carcass holding temperature.

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Table 1. Treatment description

Treatment	Description
1. S + 16°C	The sides were electrically stimulated (S) and held at 16°C for 5 hours before being subjected to a chilling temperature (2°C) for subsequent 24 hours.
2. S + 2°C	The sides were electrically stimulated (S) and immediately subjected to chilling temperature (2°) for 24 hours.
3. C + 16°C	The sides were unstimulated or control (C) and treated as in treatment 1.
4. C + 2°C	The sides were unstimulated or control (C) and held as in treatment 2.

Table 2. Muscle shortening (%), shear force (Kg) and cooking loss (%) values for ST and LD muscles as affected by electrical stimulation and mode of chilling.

Treatment[1]	Muscle shortening (%)[2]	Shear force, (kg)[3]		Cooking loss (%)[2]	
	ST	ST	LD	ST	LD
S + 16°C	10.6[a]	5.0[a]	4.1[a]	13.2[a]	19.7[a]
S + 2°C	13.1[ab]	5.6[b]	4.0[a]	16.1[a]	18.9[a]
C + 16°C	15.7[b]	6.3[c]	5.1[b]	16.3[a]	19.7[a]
C + 2°C	19.6[c]	6.4[c]	5.4[b]	14.3[a]	19.0[a]
S.D. of Adj. Mean	0.99	0.13	0.13	0.52	0.50

[1] See Table 1 for treatment.

[2] Each muscle shortening and cooking loss value is averaged from 12 samples in both ST and LD muscles.

[3] Each shear force value is averaged from 48 samples for ST muscle and from 72 samples for LD muscles.

Means within a column followed by different letters are significantly different ($P < .05$).

Table 3. Solubility of different protein fractions in LD muscle as affected by electrical stimulation and mode of chilling.

Protein fraction (%)	Treatment[1]				S.D. of Adj. Mean
	S + 16°C	S + 2°C	C + 16°C	C + 2°C	
Sarcoplasmic	3.54^{a}	3.94^{a}	4.11^{a}	3.81^{a}	0.29
Myofibrillar[2]	4.46^{b}	4.22^{b}	4.40^{b}	4.42^{b}	0.19
Myofibrillar[3]	7.13^{c}	7.04^{c}	6.91^{c}	7.00^{c}	0.18
Total Myofibrillar	11.59^{d}	11.26^{d}	11.31^{d}	11.42^{d}	0.19
Intracellular Protein	15.13^{e}	15.20^{e}	15.42^{e}	15.23^{e}	0.35
Acid Soluble Collagen	1.19^{f}	1.25^{f}	1.19^{f}	1.22^{f}	0.13
Acid Insoluble Collagen	2.77^{g}	2.55^{g}	2.47^{g}	2.63^{g}	0.45
Extracellular Protein	3.96^{h}	3.80^{h}	3.66^{h}	3.85^{h}	0.39
Total Protein	19.09^{i}	19.00^{i}	19.08^{i}	19.08^{i}	0.03
Swelling factor[4]	59.88^{j}	56.24^{j}	60.24^{j}	59.65^{j}	0.06

[1]See Table 1 for treatment.
[2]Extracted with 0.3 M NaCl in unbuffered solution.
[3]Extracted with 0.6 M KI in 0.1 M phosphate buffer.
[4]Swelling factor = $\frac{\text{Weight of the sample (drained)}}{\text{Dry weight of sample}}$

Means within each row followed by the same letter are not significantly different ($P > .05$).

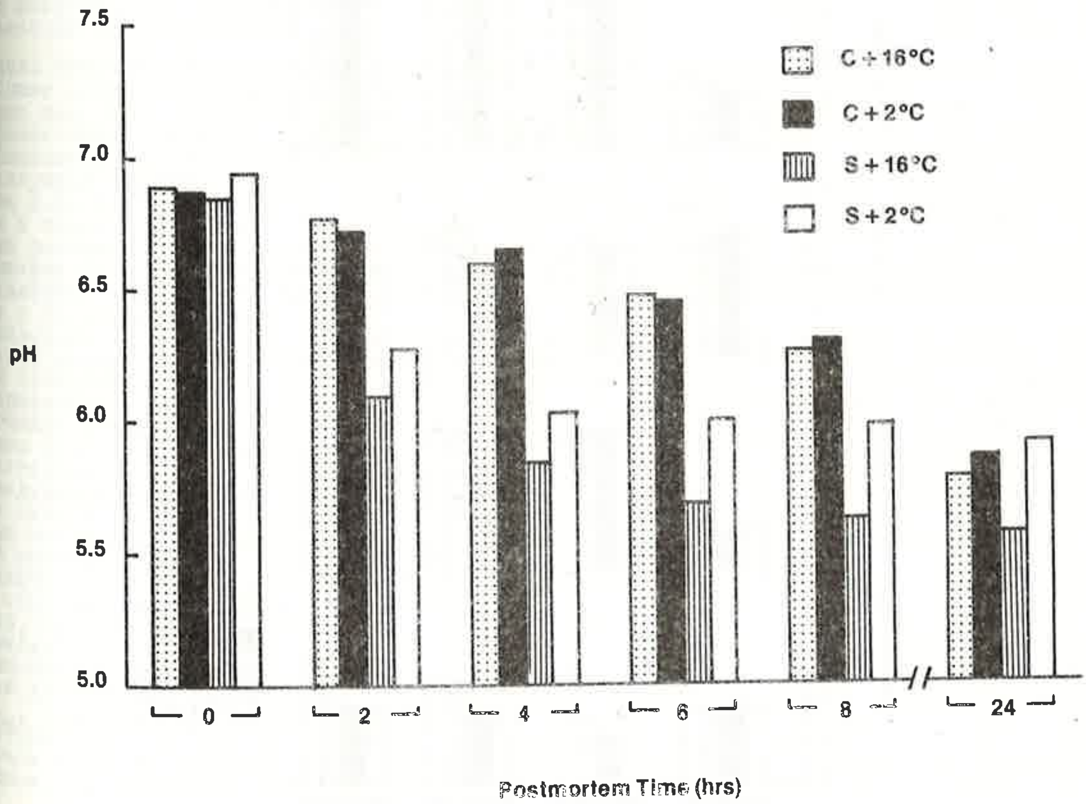

Figure 1. Postmortem pH decline for LD muscle as affected by electrical stimulation and mode of chilling.

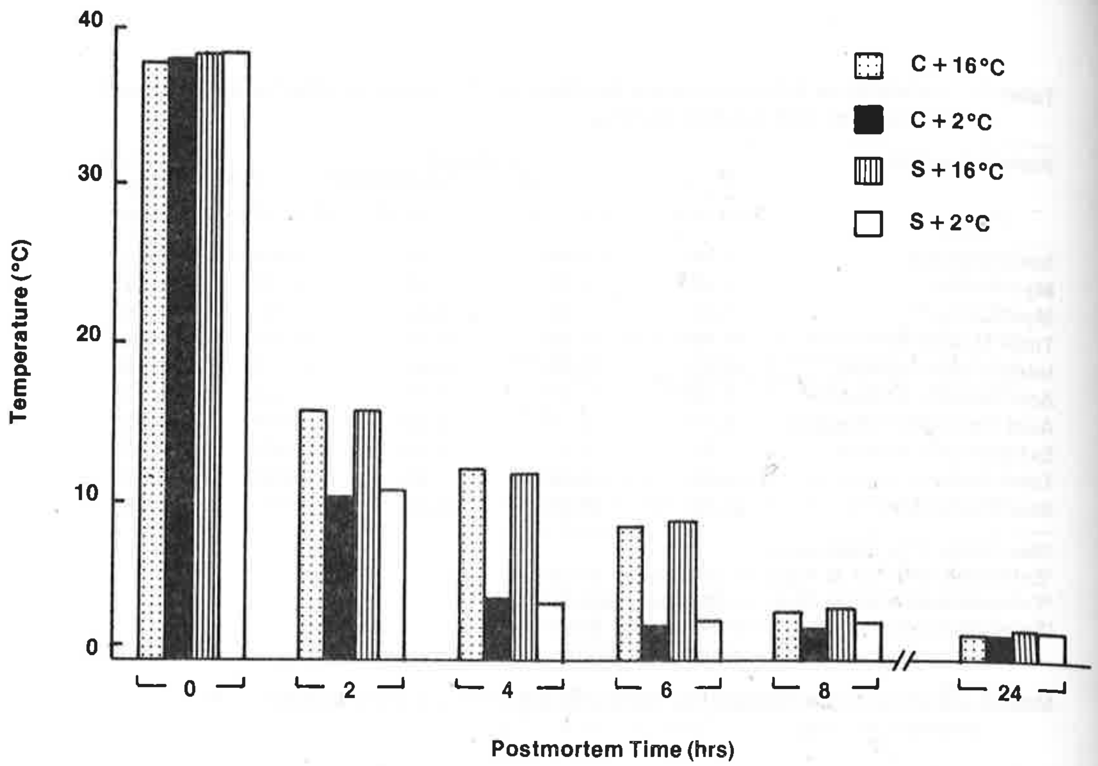

Figure 2. Postmortem temperature decline for LD muscle as affected by electrical stimulation and mode of chilling.

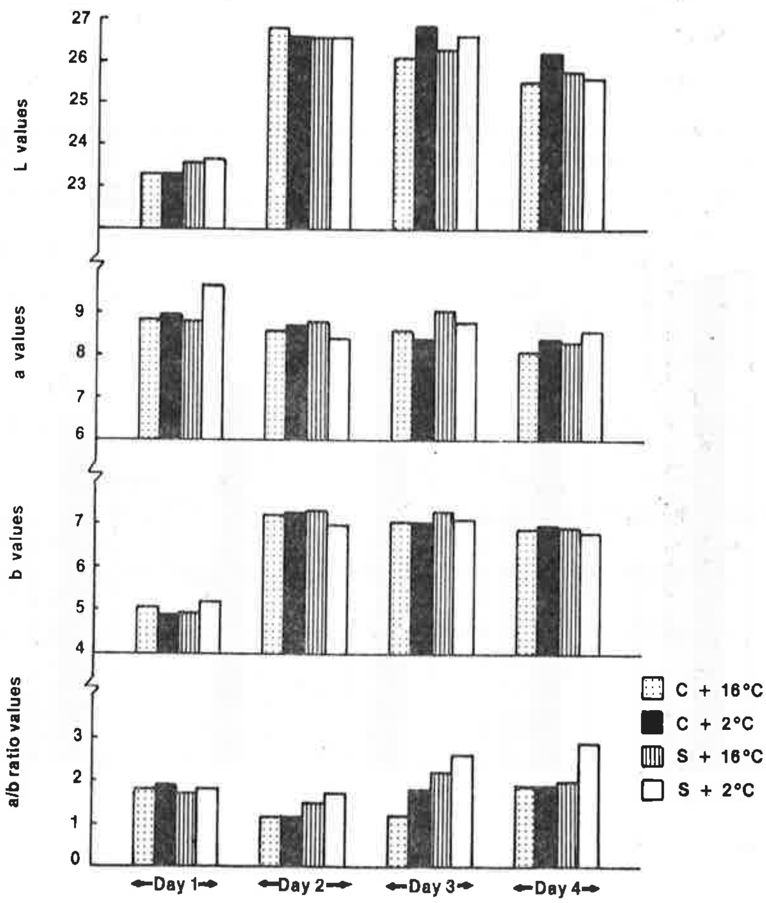

Figure 3. Hunterlab L, a, and b color values and a/b color ratio for LD muscle at day 1 to day 4 as affected by electrical stimulation and mode of chilling.