EVALUATION OF ELECTRICAL STIMULATION PARAMETERS WITH PARTICULAR REFERENCE TO BIOCHEMICAL AND QUALITY CHARACTERISTICS OF MEAT FROM LAMB

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CHAPTER I

INTRODUCTION

ELECTRICAL STIMULATION of the lamb carcass (Carse, 1973; Bendall, 1976; Chrystall and Hagyard, 1976; Whiting et al., 1981) and beef carcass (Bendall et al., 1976; McCollum and Henrickson, 1977; Shaw and Walker, 1977; Bouton et al., 1978; Chrystall and Devine, 1978; Will et al., 1979; Elgasim et al., 1981) has been shown to increase the rate of muscle postmortem glycolysis and to hasten the onset of rigor mortis. The application of these findings to the meat industry could reduce the toughening effect due to cold shortening, thaw rigor, and the post-slaughter chilling and processing period. Electrical stimulation does more than accelerate the postmortem aging process and prevent cold shortening. Stimulation increases tenderness, promotes a more rapid expression of marbling, increases muscle firmness, increases the brightness of the muscle color, facilitates USDA grading score, reduces heat-ring development and improves the appearance of meat held under retail display (Smith et al., 1977; Savell et al., 1978; McKeith et al., 1980; George et al., 1980; Riley et al., 1980).

However, there have been a lot of variations in the electrical parameters (type of current, voltage, frequency, pulse duration, pulse shape, etc.) that have been used by research workers to achieve the above mentioned benefits. In most cases, there seems to be no theoretical consideration given in selecting a particular set of electrical

stimulating parameters. For instance, voltages as high as 3600 V (Gilbert and Davey, 1976; Chrystall and Hagyard, 1976) and as low as 5 V (Enamorado, et al., 1981) and frequencies ranging from 2400 Hz (Belousov et al., 1981) to 5 Hz (Bouton et al., 1978) have been used for stimulating beef and lamb carcasses. In addition there seems to be little information available on the most appropriate combination of the electrical parameters for the stimulation of beef and lamb carcasses. It is likely that a relationship exists between the electrical stimulation parameters, especially voltage and frequency for accelerating postmortem glycolysis; however, such a relationship has not previously been studied on intact carcasses.

Even though electrical stimulation has been commercially adopted, little information is available regarding its combined effect with carcass holding temperature. Hence, the objectives of these experiments were: (1) to study the effect of different combinations of voltages and frequencies on stimulation of lamb carcasses to hasten the postmortem aging process; (2) to learn the relationship, if any, between different electrical stimulation parameters and the rate of postmortem biochemical changes in carcass with reference to meat quality, and (3) to investigate the combined effect of electrical stimulation and slow chilling of lamb carcasses on some biochemical and quality characteristics of specific ovine muscles.

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CHAPTER II

EVALUATION OF CERTAIN ELECTRICAL PARAMETERS FOR STIMULATING LAMB CARCASSES

N. H. Rashid, R. L. Henrickson, A. Asghar and P. L. Claypool

ABSTRACT

The effectiveness of electrical stimulation (ES) at two voltages (50 and 350 V) and three frequencies (10, 100, and 250 Hz) was evaluated on lamb sides. Twenty-one lambs were used. Electrical stimulation of 350 V with 10 Hz caused significantly faster postmortem glycolysis in the Longissimus dorsi (LD) and Semimembranosus muscles and less Ca^{++}-induced shortening in the Semitendinosus muscle than achieved by any other combinations of voltages and frequencies. This treatment also provided the greatest energy per pulse and caused fast and vigorous twitching of most muscles during stimulation. These results suggested that the ES parameters of high voltage with low frequency were more effective in accelerating postmortem glycolysis than low voltage with high frequency. However, the solubility of different protein factions in LD muscle was not affected by any of the experimental treatments.

INTRODUCTION

THERE is now ample experimental evidence that postmortem electrical stimulation of the carcass provides many benefits over the unstimulated carcass. This aspect has been discussed at length in recent

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reviews (Cross, 1979; Bendall, 1980; Asghar and Henrickson, 1982). However, there has been a lot of variation in the electrical parameters (type of current, voltage, frequency, pulse duration, pulse shape, etc.) that have been used by research workers. For instance, voltages as high as 3600 (Chrystall and Hagyard, 1976; Gilbert and Davey, 1976) and as low as 5 (Enamorado et al., 1981) have been used for stimulation of beef and lamb carcasses. In an earlier study, Harshman and Deatherage (1951) used 2000 to 2500 V. Gilbert (1978) suggested 1100 V, whereas Bendall et al., (1976) advocated 700 V. Savell et al. (1978) employed 440 V while McCollum and Henrickson (1977), Tang and Henrickson (1980), and Will et al. (1980) used 300 V. Golovkin et al. (1981) reported 15 V to be the optimum voltage for stimulation of muscle. Since the use of high voltages required major safety considerations in a commercial abattoir, some researchers were led to examine the effectiveness of low current ranging from 5 - 100 V for accelerating postmortem glycolysis while maintaining meat quality (Westervelt and Stouffer, 1978; Bouton et al., 1978; Taylor and Marshall, 1980).

With regard to the frequency (Hz) of electrical current, Belousov et al. (1981) used as high as 2400 Hz, and Bouton et al. (1978) employed as low as 5 Hz for stimulation of the carcasses. Harshman and Deatherage (1951) preferred to keep the frequency close to 60 pulses per second for stimulating beef carcasses, while Golovkin et al (1981) suggested 25 Hz. Henrickson and associates have used 400 Hz (McCollum and Henrickson, 1977; Tang and Henrickson, 1980; and Will et al., 1980). Chrystall and Devine (1978) compare the effect of different frequencies on excised muscles and recommended 5 - 16.5 Hz to achieve the greatest

pH fall. It is likely that there is a relationship between the electrical parameters (i.e., voltage and frequency) for accelerating postmortem muscle glycolysis. However, such a relationship has not previously been studied on the intact carcasses. Hence, this study was conducted to investigate the effect of electrical stimulation of lamb carcasses at different voltage and frequency levels on some biochemical and quality characteristics of lamb muscle.

MATERIALS AND METHODS

Experimental design

This study involved two voltage levels (50 and 350 V) with three frequency levels (10, 100 and 250 Hz) at each voltage. Altogether, there were seven treatments including the control. Twenty-one Suffolk wether lambs were randomly assigned to 21 blocks, and two treatments were randomly assigned to each block. The two treatments were randomly assigned to the two carcass sides within a block. The animals were randomly removed from the lot and slaughtered in the Meat Science Abattoir at Oklahoma State University, skinned, eviscerated, and divided into sides for experimental treatment (carcass weight ranged from 19 to 25 kg). Thereafter, each side was transferred into a temperature-controlled room at $14\pm2^{o}C$ where electrical stimulation was applied. After stimulation, the sides were kept at the same temperature until the pH of the longissimus dorsi (LD) and semimembranosus (SM) muscles reached 6.0, then the sides were subjected to chilling temperature at $2^{o}C$ until 24 hours postmortem.

Electrical stimulation

The sides received the electrical stimulation treatment as

appropriate within 15 minutes postmortem. The electrical current was applied by two wires each terminating with a spring-loaded jaw type clamp. One clamp was attached to the neck near the 5th and 6th cervical vertebrae as the negative charge, whereas the other clamp was attached to the achilles tendon as the positive charge (near its muscular attachment). The sides were hung from the achilles tendon using a track roller so that the rail served as further grounding to complete the circuit. A direct current with a square wave pulse at the desired voltage and frequency was applied to the carcass side for four minutes. The duty cycle was kept constant at 20% in every case. Both voltage and duty cycle were adjusted by using a Dual-Beam Oscilloscope, Type 502, while the frequency was recorded by a BK-Precision Type 1801, Frequency Counter. The resistance of two carcass sides was measured within fifteen minutes postmortem to determine the energy output during stimulation, using a Simpson Digital Multimeter Model 464. The physical response of the carcass sides (initial responses of the side, twitching of the muscles and duration of twitching) was noted by visual observations during electrical stimulation.

Muscle sampling

Samples from three muscles, namely, LD, SM, and semitendinosus (ST), were taken post-stimulation to measure physico-chemical changes as described below.

Muscle pH. Sample cores (1.27 cm in diameter) were taken from the intact LD muscle (at level of 9th and 13th thoracic vertebrae) and from the SM muscle at 0, 2, 4, and 24 hr post-stimulation intervals. Additional sample cores were also taken at 30-minute intervals (when the

pH values were >6.1) and at 15-minute intervals (when the pH values were <6.1) from both LD and SM muscles to determine the time required to reach pH 6.0. A 1.5 g sample (taken from the center of the core) was homogenized with 15.0 ml of 0.005 M sodium iodoacetate (to arrest glycolysis) for 30 seconds in a Brinkmann Polytron (Nichols and Cross, 1980). The pH of the slurry was measured with a Digital Corning-130 pH Meter.

Muscle temperature. The changes in the internal muscle temperature of the intact LD and SM were measured with a temperature probe, Koch Model 1364, at 0, 2, 4, and 24 hr post-stimulation intervals.

Ca^{++}-induced shortening. The ST muscle was removed from the carcass side immediately after electrical stimulation and divided longitudinally into four strips of approximately equal weight. The initial length of each strip was marked by inserting straight pins in either end. Two strips selected at random were injected with 0.1 M $CaCl_2$ (1 ml/100 g muscle) while the other two strips were injected with equivalent amounts of deionized water (controls). The total dosage was administered in six to eight increments in both deep and superficial tissue. The strips were placed in a deep tray, covered with Handi-W food wrap (Dow Chemical Company, Midland, Michigan) to guard against evaporation, and placed in a cold room at $2^{o}C$. After 24 hours, the final length of each strip was measured and the percent shortening was calculated. The shortening caused by $CaCl_2$ injected was referred to as Ca^{++}-induced shortening (Pearson et al., 1973, and Asghar et al., 1981).

Protein solubility. The LD muscle adjacent to the 3rd and 8th thoracic vertebrae was removed from the sides at 24 hours postmortem to study the solubility of different proten fractions. Each

sample was prepared following the procedure of Asghar and Yeates (1974). Triplicate, 2 g, samples from the homogenous minced meat were extracted sequentially with different buffer systems using protein extraction apparatus as described by Asghar and Yeates (1974). The sarcoplasmic proteins were extracted with 2% glycerol solution (Scopes, 1968). The residue was extracted with 0.3 M KCl in 0.1 M phosphate buffer (pH6.4) to isolate myofibrillar proteins and then with 0.6 M Kl in 0.1 M tris-buffer (pH 7.2) to isolate the remaining myofibrillar proteins (Asghar et al., 1981). All extractions and centrifugation were performed at 2° C. The resulting residue was washed thoroughly with deionized water, extracted with chloroform-methanol (3:1, v/v) to remove the lipids, and dried at 105°C. The dried residue has been referred to as connective tissue (Asghar and Yeates, 1974) although it does contain desmin and some actin (Lazarides and Hubbard, 1976). The protein content in different extracts was measured by the biuret reaction and the A 540 nm was determined using a Gilford Spectrophotometer (Gronall et al., 1949).

Statistical analysis

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

RESULTS AND DISCUSSION

Physical response of carcass sides

The physical response of lamb carcass sides during electrical

stimulation of different voltages and frequencies are shown in Table 1. It was noted that cervical and thoracic regions of the sides bend more vigorously in a lateral direction when stimulated at a high voltage (350 V) than at a low voltage (50 V). It was also observed that the frequency had a pronounced effect on the extent of muscle twitching. Most of the muscles on the carcass surface exhibited fast twitching during stimulation at the low frequency (10 Hz) regardless of whether the voltage was low or high; however, twitching continued for a longer period at the low voltage than at the high voltage. The twitching of muscles was apparent at 100 Hz in a few muscles such as in the leg and neck region. At 250 Hz, the whole side went into a tetanic condition without showing any twitching of individual muscles. This condition occurred independently of voltage.

Time required to reach pH 6.0

How fast the postmortem muscle pH drops to 6.0 has been used as a criterion to determine the effectiveness of electrical stimulation (Chrystall and Devine, 1978; Chrystall et al., 1980). Table 2 shows that both voltage and frequency had a tremendous effect on the time required for the muscle to reach 6.0 when stimulating carcass sides. The data indicated significant differences in time for the LD ($P < 0.005$ and SM ($P < 0.05$) muscles to reach pH 6.0 when carcass sides were stimulated at 350 V and 50 V, while the frequency was varied from 10 to 250 Hz. Stimulation at 350 V with 10 Hz required the shortest period of time for the muscles to reach pH 6.0. For instance, the LD and SM muscles attained pH 6.0 in 3.91 hr and 3.95 hr, respectively, whereas the control sample of the same muscles took 12.22 hr and 11.22 hr,

TABLE 1

PHYSICAL RESPONSE* OF LAMB CARCASS SIDES DURING ELECTRICAL STIMULATION OF DIFFERENT VOLTAGES AND FREQUENCIES WITHIN 15 MINUTES AFTER BLEEDING FOR FOUR MINUTES

Electrical Parameters		Initial Responses of the Carcass Side**	Twitching of Muscles	Duration of Twitching (sec.)
Voltage (V)	Frequency (Hz)			
50	10	Moderate	Fast twitching of most of the muscles	180 - 240
50	100	Moderate	Very slight twitching in few muscles	60 - 120
50	250	Moderate	No obvious twitching of muscles	-
350	10	Vigorous	Fast and vigorous twitching of most muscles	120 - 180
350	100	Vigorous	Very slight twitching in few muscles	20 - 80
350	250	Vigorous	No obvious twitching of muscles	-

*Based on visual observation.
**The cervical and thoracic regions bend laterally outward.

TABLE 2

TIME REQUIRED TO REACH pH 6 FOR LD AND SM MUSCLES AS AFFECTED BY ELECTRICAL STIMULATION AT DIFFERENT VOLTAGES (V) AND FREQUENCIES (Hz)

ES Treatment		Time required to reach pH 6.0 (Hr)[1]	
Voltage (V)	Frequency (Hz)	LD Muscle	SM Muscle
50	10	8.91^{bc}	8.47^{c}
50	100	8.72^{c}	8.70^{bc}
50	250	10.07^{b}	9.77^{b}
350	10	3.92^{e}	3.91^{d}
350	100	7.12^{d}	7.81^{c}
350	250	7.97^{cd}	8.59^{c}
Unstimulated (control)		12.33^{a}	11.22^{ac}
SD of Adj. Mean		0.39	0.37

[1]Each value of time required to reach pH 6.0 is averaged from six samples in both LD and SM muscles.

Means within a column followed by different letters are significantly different ($P<0.05$).

respectively. Other stimulation treatments required more time for the muscles to reach pH 6.0 than stimulation at 350 V with 10 Hz, but they were still significantly less than the control for both the LD ($P<0.005$) and SM ($P<0.005$) muscles. Carse (1973) found that stimulating lamb with 250 V caused the pH to decline to 6.0 in approximately three hours, while Chrystall and Hagyard (1976) reported that 3600 V reduced the pH to 6.0 in less than one hour. In this study, high voltage (350 V) had a greater effect in reducing the time to reach pH 6.0 than a low voltage (50 V) for both the LD ($P<0.005$) and SM ($P<0.005$) muscles regardless of the frequency. This agrees with earlier findings of several workers (Carse, 1973; Bendall et al., 1976; Bendall, 1980; Bouton et al., 1980), who have shown that stimulation at high voltage causes a faster drop in muscle pH. High voltage also uniformly accelerated the biochemical reactions throughout the carcass (Chrystall and Devine, 1978; Chrystall et al., 1980; Bouton et al., 180). Apart from this, the present study showed that the frequency was also an important factor in reducing the time for the muscle to reach pH 6.0. The data in Table 2 show that a low frequency was more effective in accelerating the glycolysis in both LD and SM muscles ($P<0.005$) than a high frequency. Thus the present study provides further evidence that frequency along with voltage increases the rate of glycolysis in muscle due to stimulation.

Energy output during stimulation

The data on energy output in lamb carcass sides during electrical stimulation at different voltages and frequencies are shown in Table 3. It can be seen that stimulation of the side with 350 V resulted in a much higher total energy output than that at 50 V. A change in

TABLE 3

THE TOTAL ELECTRICAL ENERGY OUTPUT AND JOULES PER PULSE OF VARIOUS ELECTRICAL PARAMETERS FOR STIMULATION OF LAMB SIDES WITHIN 15 MINUTES AFTER BLEEDING FOR FOUR MINUTES

ES Parameters			Total electrical energy output (KJ)	Joules/ pulse (J/P)
Voltage (V)	Frequency (Hz)	Duty Cycle (DC) %		
50	10	20	0.41	0.17
50	100	20	0.41	0.02
50	250	20	0.41	0.01
350	10	20	21.84	9.10
350	100	20	21.84	0.91
350	250	20	21.84	0.63

frequency from 10 to 250 Hz had little effect on the total energy output. The output energy per pulse decreased as the frequency was increased. The decrease in joules per pulse was also affected by voltage. For instance, electrical stimulation at 350 V with 10 Hz provided the greatest energy output per pulse (9.1 J/P); thus, the energy per pulse was markedly lower at 50 V and further decreased with an increase in frequency. There seems to be a relationship between the output energy per pulse and the extent of postmortem glycolysis.

The electrical stimulation at 350 V with 10 Hz resulted in both the least time for the muscle to reach pH 6.0 and the highest output energy per pulse (see Table 2 and Table 3). Also, based on visual observations, the treatment of 350 V with 10 Hz showed fast and vigorous twitching of most muscles in the carcass during electrical stimulation which possibly converts more chemical energy at the expense of adenosine triphosphate (ATP) in the muscle into mechanical energy of movements as compared to other stimulation treatments. Moreover, a high correlation has also been reported between ATP and lactate content, which can be reinterpreted in terms of pH value precisely (Bendall, 1976). Hence, it is quite possible that the output energy per pulse at certain electrical stimulation parameters acts as a governing factor in determining the rate of muscle postmortem glycolysis. More work is needed to substantiate such a proposition.

pH and temperature decline

The effect of electrical stimulation has largely been evaluated by measuring the pH changes in postmortem muscle since altering the pH indicates the extent of glycolysis (Bendall, 1973). Figures 1 and 2

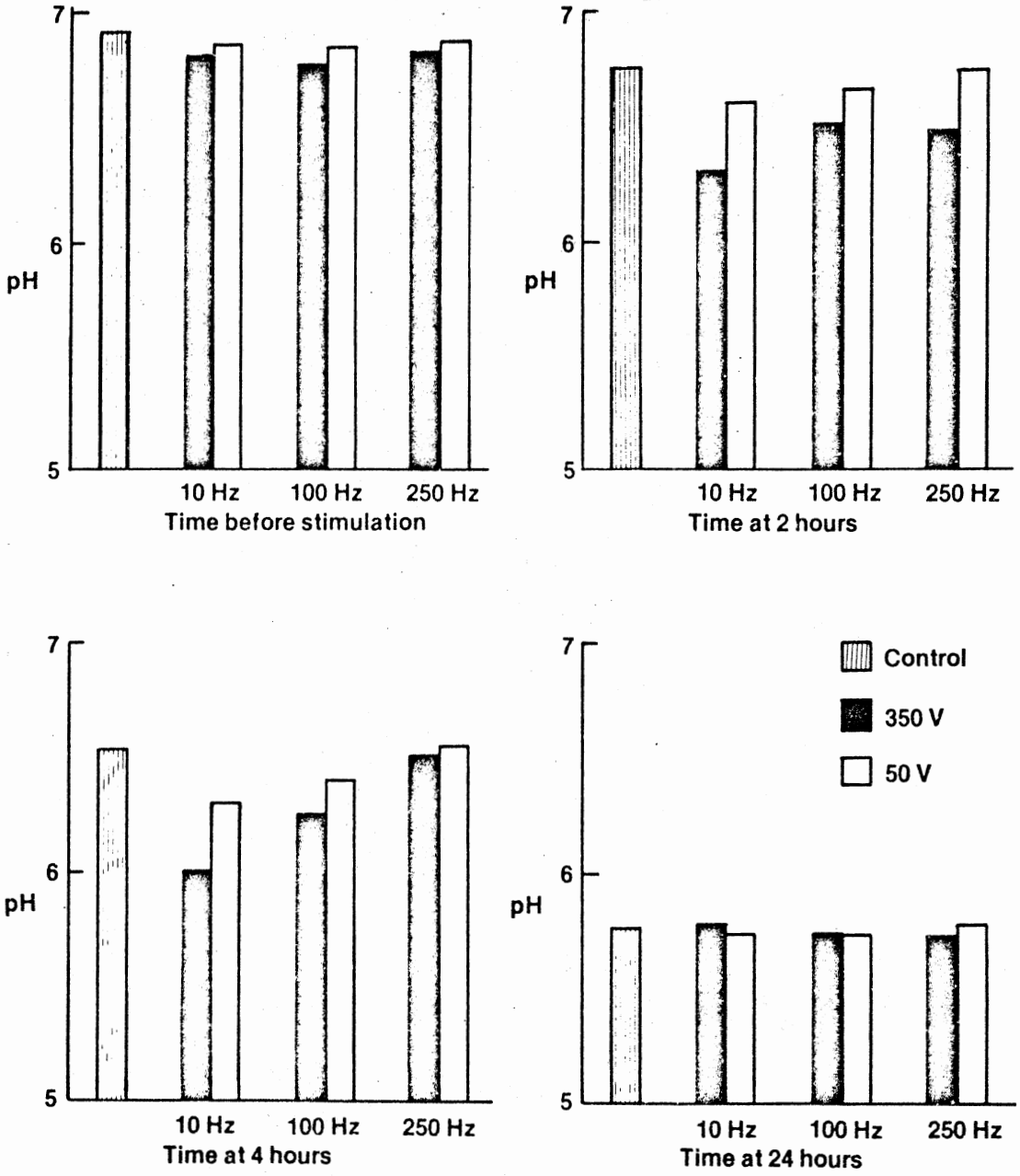

Figure 1. Postmortem pH decline for intact LD muscle before stimulation and at 2, 4 and 24 hours as affected by electrical stimulation at different voltages (V) and frequencies (Hz)

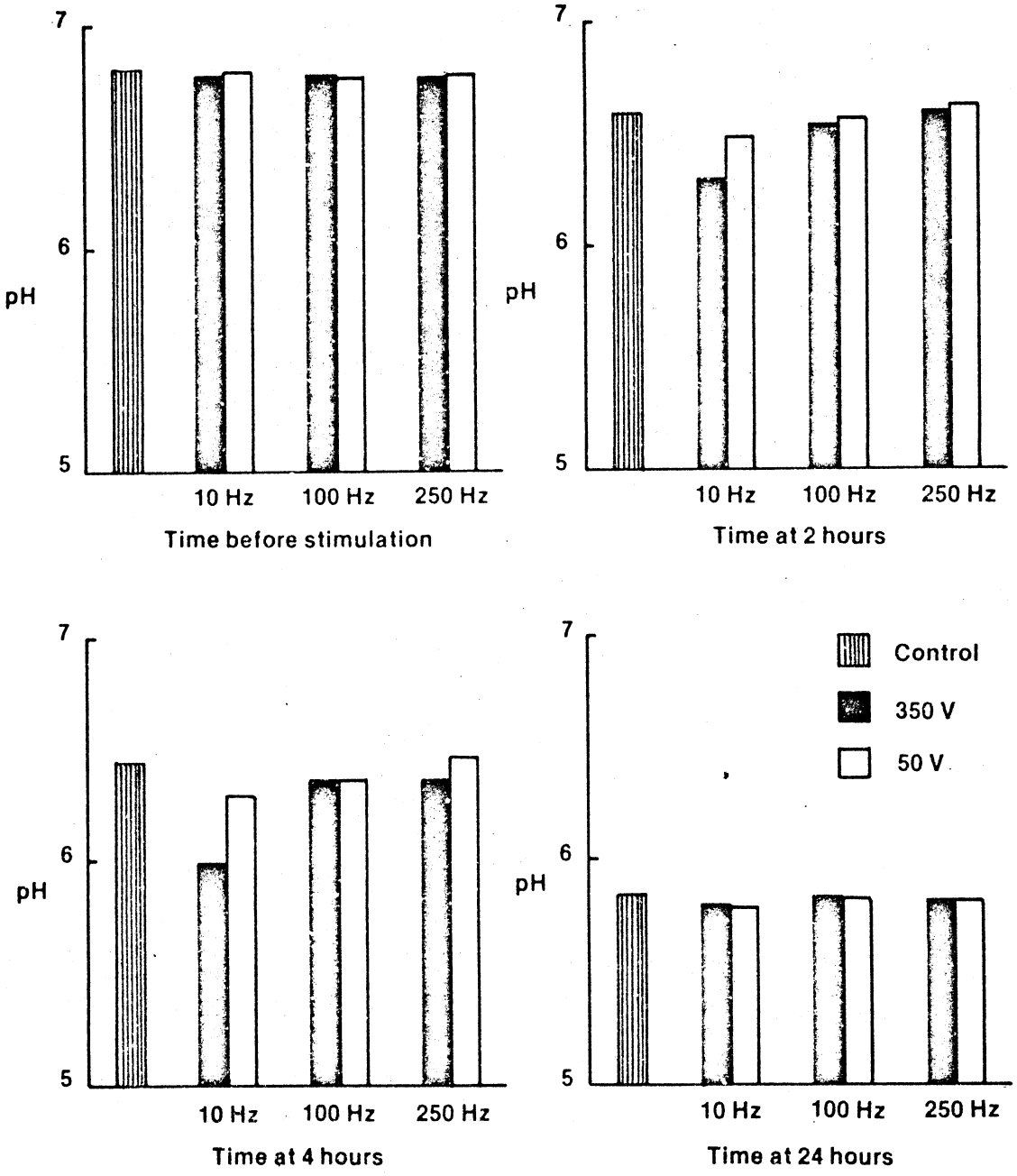

Figure 2. Postmortem pH decline for intact SM muscle before stimulation and at 2, 4 and 24 hours as affected by electrical stimulation at different voltages (V) and frequencies (Hz)

show the effect of different electrical stimulation parameters (i.e., voltage and frequency) on pH fall in the LD and SM muscles, respectively, at 0, 2, 4, and 24 hr post-stimulation. Current at 350 V with 10 Hz caused the greatest pH fall ($P<0.05$) in the LD and SM muscles at two and four hours post-stimulation period. However, at the two-hours interval, the pH of the LD muscle from lamb sides stimulated at 50 V with 10 Hz and 50 V with 100 Hz was not significantly different from that of the control treatment ($P>0.05$), while the pH of the SM muscle at the treatment of 50 V with 100 Hz, 50 V with 250 Hz, 350 V with 250 Hz and the treatment of control was also not significantly different ($P>0.05$). This study indicated that the effectiveness of a particular electrical stimulation system, when evaluated on one muscle, may not be adequate since different muscles do not respond alike. At four-hour intervals, the treatment of 50 V and 250 Hz was not significantly different from the control ($P>0.05$) for both the LD and SM muscles. This suggested that a low voltage and higher frequency were less effective and less consistent in influencing the postmortem glycolysis than that of high voltage and low frequency. At 24 hr postmortem, the ultimate pH of the muscles was not significantly different between the treatments ($P>0.05$). The pattern of postmortem temperature changes of intact LD and SM muscles is shown in Table 4. There were no significant differences at 0, 2, 4, and 24 hr post-stimulation (the same time interval as the pH measurement) among the treatments ($P>0.05$).

Ca^{++}-induced shortening

The effect of electrical stimulation of lamb sides at different voltages and frequencies on the extent of shortening of excised ST

TABLE 4

PATTERN OF POSTMORTEM TEMPERATURES (^{o}C) CHANGES FOR INTACT LD AND SM MUSCLES AT 0, 2, 4 and 24 HR WHEN THE pH MEASUREMENTS WERE DONE

ES Treatment		LD Muscle				SM Muscle			
Voltage (V)	Frequency (Hz)	0 hr*	2 hr	4 hr	24 hr	0 hr*	2 hr	4 hr	24 hr
50	10	37.4^{a}	16.7^{b}	12.6^{c}	2.8^{e}	39.8^{f}	22.7^{g}	16.0^{h}	3.1^{i}
50	100	37.3^{a}	17.1^{b}	12.9^{c}	2.7^{e}	39.6^{f}	23.5^{g}	16.7^{h}	3.0^{i}
50	250	37.3^{a}	16.8^{b}	12.9^{c}	2.7^{e}	39.9^{f}	22.1^{g}	16.5^{h}	3.1^{i}
350	10	37.5^{a}	16.9^{b}	12.7^{c}	2.6^{e}	39.6^{f}	22.9^{g}	16.0^{h}	3.1^{i}
350	100	37.6^{a}	16.6^{b}	13.3^{c}	2.7^{e}	39.6^{f}	22.9^{g}	16.5^{h}	3.2^{i}
350	250	34.4^{a}	16.2^{b}	12.8^{c}	2.7^{e}	39.8^{f}	22.1^{g}	16.0^{h}	3.1^{i}
Unstimulated (control)		37.6^{a}	16.2^{b}	12.4^{c}	2.7^{e}	39.6^{f}	23.1^{g}	16.8^{h}	3.1^{i}
SD of Adj. Mean		0.20	0.64	0.33	0.06	0.12	1.13	0.43	0.06

*0 hr = zero hour, where muscle temperatures were measured immediately before stimulation procedure.

Means within each column followed by the same letter are not significantly different (P>0.05).

muscle strips is summarized in Table 5. Electrical stimulation had a significant influence on the potential of Ca^{++}-induced shortening of muscle. High voltage (350 V) had the greatest significant effect ($P< 0.05$) in reducing the Ca^{++}-induced shortening than stimulation at a low voltage (50 V) as the frequency increased from 10 to 100 Hz and from 10 to 250 Hz. At 50 V, the frequency had no effect ($P>0.05$) on the shortening of the ST muscle. The excised ST muscle from carcass sides which were stimulated at 350 V and 10 Hz experienced the minimum shortening (19.5%) whereas the same muscle as the control exhibited the greatest percent of shortening (32.7%). The shortening of the ST muscle strips injected with deionized water shows the same trend as was observed with Ca^{++}-ions injection. The review by Asghar and Pearson (1981) indicated that Ca^{++}-ions and ATP content are the major factors which govern cold shortening of muscle. The postmortem release of Ca^{++}-ions from the sacroplasmic reticulum (Tume, 1979, 1980) and/or from the mitochondria (Cornforth et al., 1980) at the time when the ATP level in muscle is still high results in a significant level of cold shortening. However, if the Ca^{++}-ions are released after some depletion of ATP from muscle has taken place, only a minor amount of shortening will occur. It is known that electrical stimulation causes rapid depletion of ATP (Bendall, 1976; Bendall et al., 1976; Will et al., 1979) which is the primary source of energy for the cold shortening process. The present study suggested that stimulation of carcasses at 350 V with 10 Hz will result in a more rapid depletion of energy source from muscle than the other treatments.

Solubility of different protein fractions

The extent of protein solubility of different fractions for the

TABLE 5

THE EXTENT OF Ca^{++}-INDUCED SHORTENING OF ST MUSCLE STRIPS AS AFFECTED BY ELECTRICAL STIMULATION AT DIFFERENT VOLTAGES AND FREQUENCIES

ES Treatment		Shortening (%) in ST muscle[1]	
Voltage (V)	Frequency (Hz)	Injected with $CaCl_2$	Injected with H_2O
50	10	26.58[b]	13.78[bc]
50	100	26.63[b]	14.22[b]
50	250	28.35[b]	13.83[bc]
350	10	19.53[d]	8.44[d]
350	100	24.02[c]	12.46[c]
350	250	26.88[b]	14.23[b]
Unstimulated (control)		32.68[a]	16.56[a]
SD of Adj. Mean		0.70	0.48

[1]Each muscle shortening value is averaged from 12 samples.

Means within a column followed by different letters are significantly different ($P<0.05$).

LD muscle from electrically stimulated lamb sides is shown in Table 6. The data show that none of the treatments had any significant effect ($P>0.05$) on the solubility of sarcoplasmic protein fractions. No change was also noted for the protein fraction, extracted with 0.3 M KCl in 0.1 M phosphate buffer and with 0.6 M Kl in 0.1 M tris-buffer sequentially. The total percentages of myofibrillar proteins, intracellular proteins, stroma residue and total proteins of muscle were not significantly different ($P>0.05$). Many researchers have reported that cold shortening and the accompanying toughness (due to ultimate actomyosin configuration) can be avoided when muscle pH has reached a value of 6.0 before subjecting the muscles to cold temperature (Bendall, 1975; Asghar and Pearson, 1981). In view of this theory, such consideration was undertaken in designing the present study to eliminate the influence of chilling temperature on the muscle protein structure, particularly myofibrillar proteins, while preserving the effect of ES on the muscle proteins. If such an assumption is true, the effect of ES at different voltages and frequencies showed no influence on the gross solubility of the major protein fractions. If such an assumption is not true, the present study suggested that neither the ES nor the combined effect of ES and chilling temperature exhibited no effect on the solubility of the different protein fractions. This is partly substantiated by the earlier observations of McKeith et al. (1980) and Whiting et al. (1981), who found no measurable differences in the solubility of the myofibrillar protein of muscle from electrically stimulated and unstimulated steer and lamb carcasses, respectively.

TABLE 6

THE EFFECT OF ELECTRICAL STIMULATION AT DIFFERENT VOLTAGES (V) AND FREQUENCIES (Hz) ON THE SOLUBILITY OF DIFFERENT PROTEIN FRACTIONS IN LD MUSCLE

Protein Fraction (%) \ ES Treatment	50 V			350 V			Control	SD of Adj. Mean
	10 Hz	100 Hz	250 Hz	10 Hz	100 Hz	250 Hz		
Total Protein	20.02^{g}	19.91^{g}	19.87^{g}	20.00^{g}	19.87^{g}	19.93^{g}	19.97^{g}	0.08
Extracellular	2.26^{f}	2.48^{f}	2.51^{f}	2.85^{f}	2.76^{f}	2.79^{f}	2.83^{f}	0.17
Intracellular	17.76^{e}	17.43^{e}	17.36^{e}	17.15^{e}	17.11^{e}	17.14^{e}	17.14^{e}	0.05
Total Myofibrillar	13.11^{d}	12.86^{d}	12.86^{d}	12.81^{d}	12.86^{d}	12.87^{d}	12.79^{d}	0.03
Myofibrillar[1]	4.59^{c}	4.99^{c}	5.42^{c}	5.16^{c}	4.75^{c}	4.74^{c}	5.02^{c}	0.28
Myofibrillar[2]	8.52^{b}	7.87^{b}	7.44^{b}	7.65^{b}	8.11^{b}	8.13^{b}	7.77^{b}	0.42
Sarcoplasmic	4.65^{a}	4.57^{a}	4.50^{a}	4.34^{a}	4.25^{a}	4.27^{a}	4.35^{a}	0.12

[1]Extracted with 0.3 M KCl in 0.1 M phosphate buffer (pH 6.4).

[2]Extracted with 0.6 M KI in 0.1 M tris buffer (pH 7.2).

Means within each row followed by the same letter are not significantly different (P>0.05).

CONCLUSIONS

THE CARCASS SIDES which were electrically stimulated with 350 V and 10 Hz exhibited significantly fastest postmortem glycolysis (LD and SM muscle) and a less Ca^{++}-induced shortening (ST muscle) than achieved by any other combination of voltage and frequency. Also, the electrical treatment of 350 V with 10 Hz provided the greatest energy per pulse and showed fast and vigorous twitching of most muscles in the side during electrical stimulation. Hence, at certain electrical parameters, it is quite possible that the output of energy per pulse acts as a governing factor in determining the rate of muscle postmortem glycolysis and possibly converts more chemical energy at the expense of ATP into mechanical energy and motions. More work is needed to substantiate such a proposition. The present study also provided further evidence that the interaction between voltage and frequency has a significant influence in the glycolytic rate in electrically stimulated carcasses. However, there seems to be a linear increase in the rate of glycolysis with a decrease in pulse frequency from 250 to 10 Hz. Therefore, the optimum frequency may be less than 10 Hz to achieve the maximum rate of glycolysis in muscles by electrical stimulation.

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CHAPTER III

BIOCHEMICAL AND QUALITY CHARACTERISTICS OF OVINE MUSCLES AS AFFECTED BY ELECTRICAL STIMULATION, HOT BONING, AND MODE OF CHILLING

N. H. Rashid, R. L. Henrickson, A. Asghar and P. L. Claypool

ABSTRACT

The combined effects of electrical stimulation and carcass holding temperature were evaluated on some biochemical and quality characteristics of intact and hot-boned ovine muscles. Twenty-four lamb sides were randomly assigned to four treatments. Electrical stimulation was performed within 15 minutes postmortem (350 V with 10 Hz) for four minutes. Electrically stimulated and slowly chilled (five hr at 14±2° C) sides exhibited significantly more rapid pH decline in the longissimus dorsi (LD) muscle, less cold shortening in the semitendinosus (ST) muscle and greater tenderness in both LD and ST muscles than sides at 2°C. None of the treatments had any effect on cooking loss in ST and LD muscles, lean color of LD muscle during a four-day retail display, and solubility of different protein fractions as well as the swelling factor of the stroma protein of LD muscles.

INTRODUCTION

HIGH TEMPERARATURE 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

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shortening will cause muscle toughness when lamb (Marsh 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 20°C until pre-rigor changes in the muscle are near completion since minimum shortening occurs in 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 of 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 carcass 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, 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 after four hours postmortem when lamb sides were electrically stimulated at 350 volts (V), 10 pulses (Hz) and 20 percent duty cycle (DC). Some of the biochemical-biophysical changes which 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 lamb carcasses at 14±°C for five hours postmortem on some biochemical and quality characteristics of specific ovine muscles.

MATERIALS AND METHODS

Animal and experimental design

Twelve Suffolk wether lambs (hot dressed carcass weight ranged from 21 to 29 kg) were slaughtered according to commerical practices in the Abattoir of the Meat Laboratory at Oklahoma State University, skinned, eviscerated and divided into sides. The two sides within each carcass were randomly assigned to two different treatments and a balanced incomplete block design, block size 2, was used. Accordingly, a total of 12 sides, selected at random, were electrically stimulated (ES) while 12 sides were kept as unstimulated (US) or control. In each case, six sides, at random, received a rapid chilling (RC) treatment and the other six sides were subjected to slow chilling (SC) as shown in Table 1.

Electrical stimulation

The sides were electrically stimulated within 15 minutes postmortem using a direct current with a square wave pulse for four 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

TABLE 1

TREATMENT DESCRIPTION

Treatment	Number of Sides	Description
1. Electrical Stimulation + Slow Chilling (ES + SC)	6	The sides were electrically stimulated and held at 14$\pm$ 2°C for five hours (slow chilling) before being subjected to a chilling temperature (2°C) for subsequent 19 hours
2. Electrical Stimulation + Rapid Chilling (ES + RC)	6	The sides were electrically stimulated and immediately subjected to a rapid chilling temperature (2°C) for 24 hours
3. Unstimulated + Slow Chilling (US + SC)	6	The sides were unstimulated (control) and treated as in treatment 1
4. Unstimulated + Rapid Chilling (US + RC)	6	The sides were unstimulated (control) and held as in treatment 2

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 biochemical and quality characteristics. The ST muscle was hot-boned from both the ES and US sides immediately after electrical stimulation, and the LD muscles remained attached to the skeleton. The extent of cold shortening and cooking loss on hot-boned ST muscle were determined at 24 hour postmortem. Postmortem pH and temperature changes were monitored on intact LD muscles for 24 hours postmortem. Thereafter, fresh samples were taken to measure the lean color, protein solubility, and cooking loss. The shear force value for both LD and ST muscles was determined at 48 hour postmortem.

Muscle pH. Sample cores (1.27 cm in diameter) were taken from intact LD muscles (at levels of 9th and 13th thoracic vertebrae) at 0, 2, 4, 6, 8, and 24 hours postmortem and 1.5 g samples (taken from the center of the cores) were immediately homogenized with 15 ml of 0.005 M sodium iodoacetate (Nichols and Cross, 1980) for 30 seconds using a Brinkman Polytron homogenizer. 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 invervals as for the pH measurements.

Muscle shortening. The ST muscle was divided longitudinally into

two strips of approximately equal weight and length. The initial length of each strip was marked by inserting straight 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 length of each strip was measured to calculate the percent shortening.

Cooking loss. Chops from the LD (3.8 cm in diameter) at the level of 1st and 3rd lumbar vertebrae and ST strips were cooked to an internal temperature of 70°C in a convection (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 2°C for 24 hours to provide equalized firmness to ensure uniform cores (Kastner and Henrickson, 1969). Three cores (1.27 cm in diameter) were taken from the LD chops (lateral, dorsal and medial), and two cores (1.27 cm in diameter) were obtained from the ST strips parallel to the direction of fibers using a coring device with an 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 Maching (Instron Corp., Model 1132). The drive and chart speeds were calibrated at 10 cm/min.

Lean color. Boneless loin chops were cut from the sides at the

level of the 3rd and 6th lumbar vertebrae and allowed to bloom for 45 minutes. They were then placed on a plastic foam tray, wrapped in oxygen permeable commercial type film and placed in a retail case at 2° C under 70-ft candles fluorescent light for four days. Hunterlab L, a and b values which indicated respectively the lightness, redness and yellowness were measured at 24 hr intervals using Hunterlab Tristimulus Colorimeter Model D25 L-9. The ratio of redness to yellowness (a/b) was also calculated.

Protein solubility. The solubility of different protein fractions was determined 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 sacroplasmic proteins were extracted with 2% glycerol solutopm (Scopes, 1968). The residue was extracted with 0.3 M NaCl unbuffered solution to dissolve myofibrillar protein and then with 0.6 M Kl in 0.1 M phosphate buffer (pH 7.2) to extract the remaining myofibrillar proteins. All extractions and centrifugations were performed at 2°C. 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 protein. Finally, the remaining residue was again washed with deionized water, dried at 105°C overnight, and designated as acid-insoluble stromal proteins. The swelling factor was also estimated according to the procedure of 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 analysis of variance using a balanced incomplete block design, block size 2. The F-test was used to determine if significant differences occurred 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 glycolysis (Figure 1). Stimulated sides, whether rapid or slow chilled, had a significantly ($P< 0.05$) lower pH than the respective control muscles at 2, 4, 6 and 8 hr postmortem. This is in agreement with various researchers who have shown that electrical stimulation acclerates the rate of postmortem glycolysis (Carse, 1973; Bendall et al., 1976; Davey et al., 1967a; McCollum and Henrickson, 1977; Chrystall and Devine, 1978; Will et al., 1978; Whiting et al., 1981). However, muscle from electrically stimulated slow chilled sides (ES + SC) experienced a greater pH decline ($P<0.05$) than the rapid chilled (ES + RC) sides. On the other hand, postmortem pH decline in the unstimulated sides whether slow or rapid chilled (US + SC and US + RC) was almost identical.

The internal temperature of the LD muscle at 2, 4, and 6 hr postmortem of stimulated (ES + RC) and unstimulated (US + RC) sides which were rapidly chilled was lower than the stimulated (ES + SC) and unstimulated (US + SC) sides which were slow chilled (Figure 2). However, there was no significant variation in temperature decline between

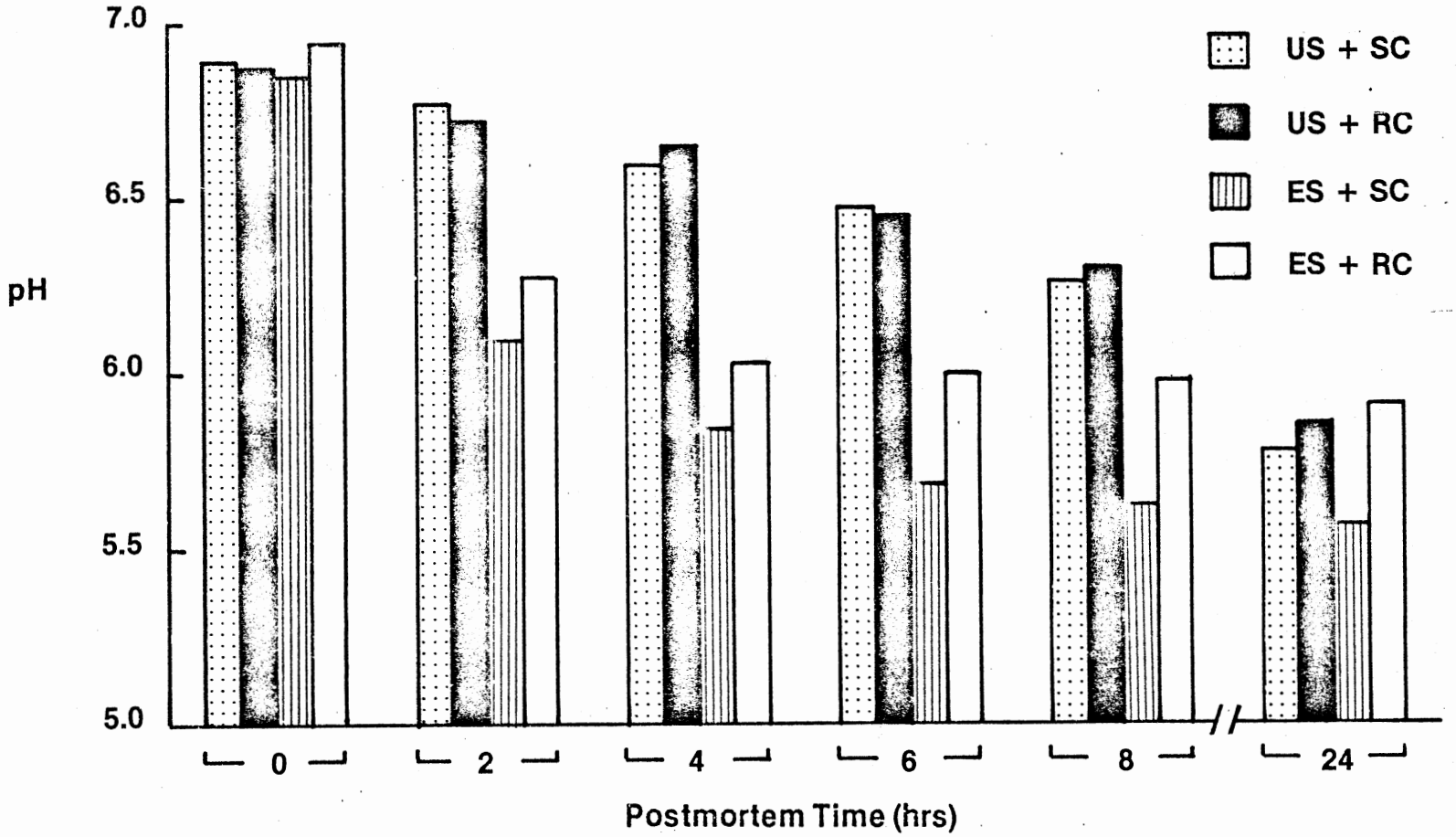

Figure 1. Postmortem pH decline for LD muscle as affedted by electrical stimulation and mode of chilling

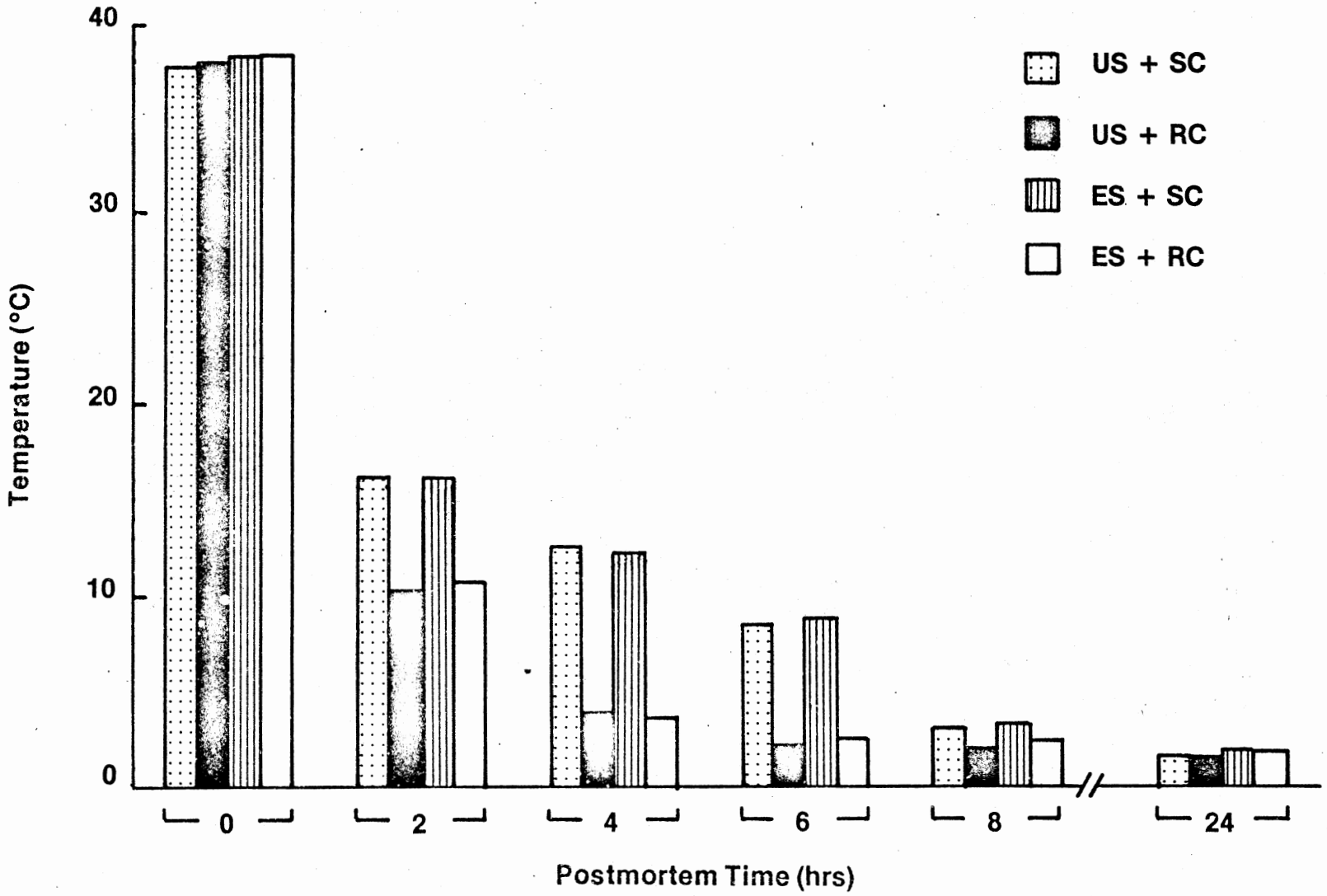

Figure 2. Postmortem temperature decline for LD muscle as affected by electrical stimulation and mode of chilling

the stimulated and control sides for any given chilling procedures (rapid or slow). Hence, differences in the rate of pH decline for the LD muscle between stimulated and control cannot be ascribed to difference in carcass temperature decline as 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. This explanation is supported by Clark et al (1980) who found that phophofructokinase, aldolase, glyceraldehyde 3-phosphate dehydrogenase and pyruvatekinase bound to actin filaments in electrically stimulated muscles and accelerated glycolytic rate. Whether or not electrical stimulation increased the glycolytic enzymes activities per se in muscle has not been completely defined.

Muscle shortening

Both electrical stimulation and mode of chilling significantly reduced cold shortening in the hot-boned ST muscle (Table 2). The muscle from stimulated and slow chiled sides (ES + SC) had significantly less ($P<0.05$) shortening than the control groups. However, there was no significant difference ($P>0.05$) 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 when rapidly chilled than when slow chilled. The present study shows that ES reduced the percent of muscle shortening. Rapid depletion of the energy rich phosphate compounds (adenosine triphosphate and phosphocreatine), which determine the degree of muscle fiber shortening during chilling or freezing of carcasses may be attributed to ES (Asghar and Henrickson, 1982). Many researchers have

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
ES + SC	10.6[a]	5.0[a]	4.1[a]	13.2[a]	19.7[a]
ES + RC	13.1[ab]	5.6[b]	4.0[a]	16.1[a]	18.9[a]
US + SC	15.7[b]	6.3[c]	5.1[b]	16.3[a]	19.7[a]
US + RC	19.6[c]	6.4[c]	5.4[b]	14.3[a]	19.0[a]
SD of Adj. Mean	0.99	0.13	0.13	0.52	0.50

[1]See Table I 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 ($P<0.05$).

reported that electrical stimulation accelerates musculature ATP depletion (Bowling et al., 1978; Will et al., 180; Whiting et al., 1981). This study supports previous reports in that, by reducing the time required for muscles to reach pH 6.0 (through the application of ES), and by holding carcasses for about five hours at $14\pm2^{o}C$, the extent of cold shortening was reduced as compared to carcasses conventionally chilled at $2^{o}C$.

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 ($P<0.05$) 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 stimulation of 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; Nillson 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 (hot-boned from electrically stimulated sides) was significantly ($P<0.005$) 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 ($P>0.05$) in either 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, and b values and the a/b color ratio of LD loin chops at 24 hr intervals for four days are shown in Figure 3. The treatment X day interaction exhibited no influence ($P>0.05$) on L, a, and b color calues and a/b color ratio. The data also indicated no significant differences ($P>0.05$) in the objective Hunterlab color values of meat among all treatments. This was contrary to the finding of Riley et al. (1980a) who showed by subjective evaluation that electrical stimulation improved muscle color, decreased surface discoloration, and improved overall appearance of boneless loin chops from lambs during four 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, b, 1979) with a more youthful lean color (McKeith et al., 1981) than that from unstimulated carcasses. However, several

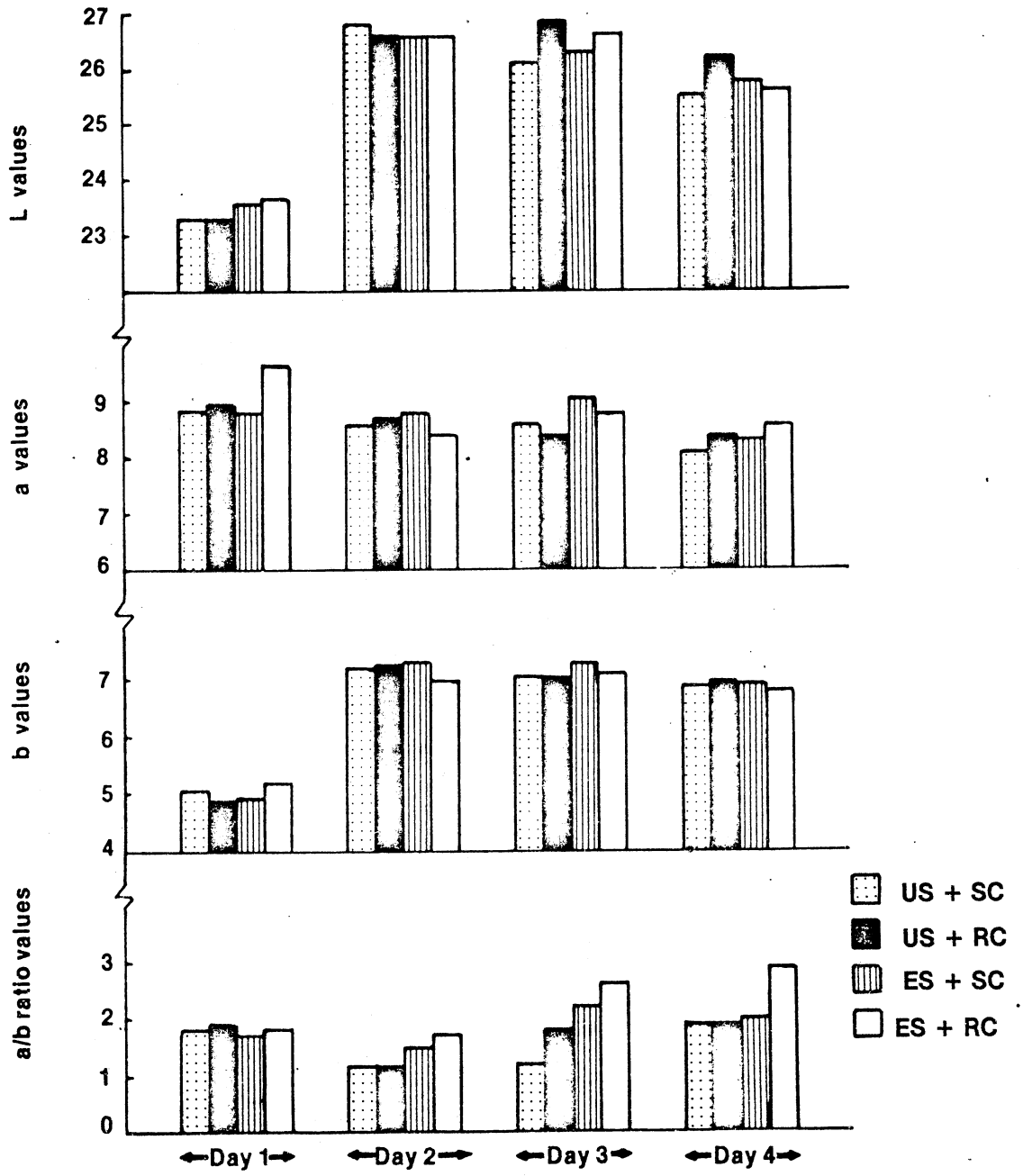

Figure 3. Hunterlab L, a, and b color values and a/b color ratio for LD muscle at day 1 to day 4 as affected by electrical stimulation and mode of chilling

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 is shown in Table 3. Neither electrical stimulation or the chilling rate had any significant effect ($P>0.05$) on the solubility of the sacroplasmic protein fraction as compared to the control. This is in disagreement with George et al. (1980) who have concluded that slow cooling of electrically stimulated carcasses causes denaturation and precipitation of sacroplasmic proteins onto the myofilbrils. If such a deposition occurs, it should be reflected in decreased sacroplasmic protein solubility. No change was noted in the solubility of myofibrillar proteins extracted sequentilly with 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 phosphate ions in the buffer dissociates the actomyosin complex and probably increases 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 formed to a different degree as a result of the different treatments applied to the carcass sides. From the solubility test with unbuffered 0.3 M NaCl solution, it seems that the different treatments had no significant effect on the extent of actomyosin formation. Similarly, the total percentage of myofibrillar protein and the intracellular protein were also not significantly different ($P>0.05$) among treatments. These observations agree

TABLE 3

SOLUBILITY OF DIFFERENT PROTEIN FRACTIONS IN LD MUSCLES AS AFFECTED BY ELECTRICAL STIMULATION AND MODE OF CHILLING

Protein Fraction (%)	Treatment[1]				SD of Adj. Mean
	ES + SC	ES + RC	US + SC	US + RC	
Total protein	19.09[a]	19.00[i]	19.08[i]	19.08[i]	0.03
Extracellular	3.96[h]	3.80[h]	3.66[h]	3.85[h]	0.39
Acid-soluble	1.19[g]	1.25[g]	1.19[g]	1.22[g]	0.13
Acid-insoluble	2.77[f]	2.55[f]	2.47[f]	2.63[f]	0.45
Intracellular	15.13[e]	15.20[e]	15.42[e]	15.23[e]	0.35
Total myofibrillar	11.59[d]	11.26[d]	11.31[d]	11.42[d]	0.19
Myofibrillar[2]	4.46[c]	4.22[c]	4.40[c]	4.42[c]	0.19
Myofibrillar[3]	7.13[b]	7.04[b]	6.91[b]	7.00[b]	0.18
Sarcoplasmic	3.54[a]	3.94[a]	4.11[a]	3.81[a]	0.29
Swelling factor[4]	59.88[j]	56.24[j]	60.24[j]	59.65[j]	0.06

[1]See Table I 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 (pH 7.2).

[4]Swelling factor = weight of dehydrated (collagen) sample/dry weight of sample.

Means within each row followed by the same letter are not significantly different (P>0.05).

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 protein (freshly synthesized collagen) and acid-insoluble stromal proteins (biologically mature collagen and some elastin) 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. Judge et al. (1980) found no increase in the solubility of the perimysial collagen from electrically stimulated muscle, but their data on different scanning colorimetry showed a significant decrease ($0.6^{o}C$) in the thermal stability of the permysial collagen of the L. dorsi muscle from electrically stimulated carcass as compared to that from the control. As a matter fact, very limited information is available on the influence of electrical stimulation of carcasses on the connective tissue (extracellular) proteins, and more information is needed.

CONCLUSIONS

THIS STUDY has shown that the combined effect of electrical stimulation and mode of chilling profoundly affect some biochemical, biophysical quality characteristics of ovine muscles. The sides which were electrically stimulated and slowly chilled (holding the carcass sides for 5 hr at $14\pm2^{o}C$) 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 either electrical stimulation or carcass holding temperature.

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CHAPTER IV

SUMMARY AND CONCLUSIONS

RECENT DEVELOPMENTS in meat science revealed that electrical stimulation of the carcass provided many quality advantages. These facts motivated the meat industry in the United States, United Kingdom and New Zealand to adopt electrical stimulation in the slaughter of both beef and lamb. However, there has been a lot of variation in the electrical stimulation parameters, especially voltage and frequency that have been used by research workers to achieve the above mentioned advantages. In addition, there seems to be little information available on the most appropriate combination of electrical parameters for stimulating beef or lamb carcasses. This study was undertaken to evaluate the effectiveness of electrical stimulation at two voltages (50 V and 350 V) and three frequencies (10, 100, and 250 Hz) on some biochemical and quality characteristics of lamb muscles, and to investigate the combined effect of electrical stimulation and mode of chilling on some biochemical changes of specific ovine muscles with reference to meat quality. In the first portion of the study, twenty-one lambs were used. The sides received the electrical stimulation treatment as appropriate within 15 minutes postmortem using direct current, pulsed as a square wave, with a 20 percent duty cycle for four minutes. The physical response of the carcass sides was noted by visual observations during electrical stimulation. The total output

energy and output energy per pulse were calculated. Temperature and pH measurements were taken at 0, 2, 4 and 24 hours post-stimulation and the time required for the muscles to reach pH 6.0 was measured. Muscle samples were taken to determine the extent of Ca^{++}-induced shortening, lean color, and solubility of different protein fractions. The second study utilized twelve animals. The stimulation procedure was similar to the first study except that the electrical parameters were fixed at 350 V, 10 Hz and 20% duty cycle. A total of twelve sides selected at random were electrically stimulated with twelve sides kept as control. In each case, six sides, at random, received rapid chilling and the other six sides were subjected to slow chilling. Temperature and pH measurements were taken at 0, 2, 4, 6, 8 and 24 hr postmortem. Muscle samples were taken to determine the cold shortening, cooking loss, shear force value, lean color during a 4-day retail display, and solubility of different protein fractions.

In the first study, sides treated with electrical stimulation at 350 V with 10 Hz significantly exhibited faster postmortem glycolysis in the LD and SM muscles and less Ca^{++}-induced shortening in the ST muscle than achieved by any other combination of voltages and frequencies. This treatment also provided the greatest energy per pulse and showed fast and vigorous twitching of most muscles during stimulation. These data led to the conclusion that the output energy per pulse possibly acted as a governing factor in determining the rate of muscle postmortem glycolysis and probably converted more chemical energy at the expense of ATP into mechanical energy and motions. This indicated, however, that the total energy was not the decisive factor in affecting the rate of glycolysis in muscle. Further research is

needed to substantiate such a proposition. The results suggested that the ES parameters of high voltage with low frequency were more effective and more consistent in accelerating the postmortem glycolysis than that of low voltage with high frequency.

The data from the second portion of the study showed that the combined effect of electrical stimulation and slow chilling (holding the carcass sides for five hours at $14\pm2^{o}C$) resulted in significantly more rapid pH decline in the LD muscle, less cold shortening in the ST muscle and greater tenderness in both LD and ST muscles as compared to the carcasses conventionally chilled at $2^{o}C$. The data for pH and temperature led to the conclusion that probably some glycolytic enzymes were activated by electrical stimulation to speed up the glycolysis. Whether or not electrical stimulation activates the enzyme system per se in muscle has not been completely defined. Further studies are needed to clarify this speculation. The differences in cooking loss among the four treatments was not significant for both LD and ST muscles. The study also showed that neither the objective lean color (L, a, and b values) nor the solubility of different protein fractions (sarcoplasmic, myofibrillar, acid-soluble protein, acid-insoluble stromal protein and the swelling factor of stroma) were influenced by the electrical stimulation and carcass holding temperature.

APPENDIXES

APPENDIX A

ANALYSIS OF VARIANCE FOR CHAPTER II

TABLE 1

ANALYSIS OF VARIANCE FOR THE TIME REQUIRED TO REACH pH 6.0 IN BOTH LONGISSIMUS DORSI (LD) AND SEMIMEMBRANOSUS (SM) MUSCLES AS AFFECTED BY ELECTRICAL STIMULATION AT DIFFERENT VOLTAGES AND FREQUENCIES

Source of Variation	d.f.	Mean Square	
		LD Muscle	SM Muscle
Total	41		
Block (Animal)	20		
Treatment (Adj.)	6	22.4595	17.7603
Control vs Others	1	55.9728***	33.6117***
Voltage	1	43.6945***	25.6489***
Frequency	2	11.8200***	16.3714***
Voltage x Frequency	2	5.7248*	7.2791***
Error	15	0.9343	0.8315

*, ***Significant at $P<0.05$ and 0.005, respectively.

TABLE 2

ANALYSIS OF VARIANCE OF pH VALUES AT 0, 2, 4 AND 24 HR POST-STIMULATION IN LONGISSIMUS DORSI MUSCLE AS AFFECTED BY ELECTRICAL STIMULATION AT DIFFERENT VOLTAGES AND FREQUENCIES

Source of Variation	d.f.	Mean Square			
		0 Hr	2 Hr	4 Hr	24 Hr
Total	41				
Block (Animal)	20				
Treatment (Adj.)	6	0.0092	0.1180	0.1579	0.0017
Control vs Others	1	0.0261	0.5640***	0.2945***	0.0003
Voltage	1	0.0195	0.3084***	0.4576***	0.0008
Frequency	2	0.0390	0.0348*	0.0854***	0.0011
Voltage x Frequency	2	0.0007	0.0085	0.0123	0.0035
Error	15	0.0065	0.0089	0.0096	0.0012

*, ***Significant at $P<0.05$ and 0.005, respectively.

TABLE 3

ANALYSIS OF VARIANCE OF pH VALUES AT 0, 2, 4 AND 24 HR POST-STIMULATION IN SEMIMEMBRANOSUS MUSCLE AS AFFECTED BY ELECTRICAL STIMULATION AT DIFFERENT VOLTAGES AND FREQUENCIES

Source of Variation	d.f.	Mean Square			
		0 Hr	2 Hr	4 Hr	24 Hr
Total	41				
Block (Animal)	20				
Treatment (Adj.)	6	0.0006	0.0398	0.0929	0.0015
Control vs Others	1	0.0010	0.0154	0.0547***	0.0030
Voltage	1	0.0006	0.0348*	0.0981***	0.0002
Frequency	2	0.0007	0.0819***	0.1505***	0.0027
Voltage x Frequency	2	0.0005	0.0124	0.0520**	0.0003
Error	15	0.0048	0.0066	0.0069	0.0027

*, **, *** Significant at $P<0.05$, 0.01 and 0.005, respectively.

TABLE 4

ANALYSIS OF VARIANCE OF TEMPERATURE VALUES AT 0, 2, 4 AND 24 HR POST-STIMULATION IN LONGISSIMUS DORSI MUSCLE AS AFFECTED BY ELECTRICAL STIMULATION AT DIFFERENT VOLTAGES AND FREQUENCIES

Source of Variation	d.f.	Mean Square			
		0 Hr	2 Hr	4 Hr	24 Hr
Total	41				
Block (Animal)	20				
Treatment (Adj.)	6	0.1429	1.5476	0.9762	0.0238
Control vs Others	1	0.0833	3.0000	2.0833	0.0000
Voltage	1	0.0119	1.7143	0.1071	0.0476
Frequency	2	0.0475	0.9643	1.5119	0.0000
Voltage x Frequency	2	0.3334	1.3215	0.3215	0.0476
Error	15	0.2429	2.4810	0.8762	0.0238

TABLE 5

ANALYSIS OF VARIANCE OF TEMPERATURE VALUES AT 0, 2, 4 AND 24 HR POST-STIMULATION IN SEMIMEMBRANOSUS MUSCLE AS AFFECTED BY ELECTRICAL STIMULATION AT DIFFERENT VOLTAGES AND FREQUENCIES

Source of Variation	d.f.	Mean Square			
		0 Hr	2 Hr	4 Hr	24 Hr
Total	41				
Block (Animal)	20				
Treatment (Adj.)	6	0.1190	6.9048	4.2500	0.0238
Control vs Others	1	0.0833	3.000	3.0000	0.0000
Voltage	1	0.1071	0.7619	0.7619	0.0476
Frequency	2	0.2262	15.2500	2.2857	0.0000
Voltage x Frequency	2	0.0357	3.5834	0.3335	0.0476
Error	15	0.0857	7.6381	1.1000	0.0238

TABLE 6

ANALYSIS OF VARIANCE OF SHORTENING OF SEMITENDINOSUS MUSCLE STRIPS INJECTED WITH 0.1 M $CaCl_2$ AND DEIONIZED WATER (CONTROL) AS AFFECTED BY ELECTRICAL STIMULATION AT DIFFERENT VOLTAGES AND FREQUENCIES

Source of Variation	d.f.	Mean Square: Injected with 0.1 M $CaCl_2$	Mean Square: Injected with Deionized Water
Total	83		
Block (Animal)	20		
Treatment (Adj.)	6	112.4823	43.3279
Control vs Others	1	324.1536***	83.5865***
Voltage	1	144.3937***	52.4094***
Frequency	2	72.6761***	32.6053***
Voltage x Frequency	2	30.4971*	29.3804***
Experimental Error	15	5.8860	2.7670
Sampling Error (Duplicate)	42	1.7834	0.9812

*, ***Significant at P<.05 and 0.005, respectively.

TABLE 7

ANALYSIS OF VARIANCE OF SARCOPLASMIC (SP), MYOFIBRILLAR EXTRACTED WITH 0.3 M KCl IN PHOSPHATE BUFFER (MF_1), MYOFIBRILLAR EXTRACTED WITH 0.6 M Kl IN TRIS-BUFFER (MF_2), TOTAL MYOFIBRILLAR (TMF), INTRACELLULAR (IC), EXTRACELLULAR (EC), AND TOTAL PROTEINS (TP) VALUES IN LONGISSIMUS DORSI MUSCLE AT 24 HR POSTMORTEN

Source of Variation	d.f.	Mean Square						
		SP	MF_1	MF_2	TMF	IC	EC	TP
Total	125							
Block (Animal)	20							
Treatment (Adj.)	6	0.0850	0.8590	1.3330	0.1142	0.5944	0.4437	0.0868
Control vs Others	1	0.0667	0.0529	0.2844	0.0920	0.3155	0.6615	0.0633
Voltage	1	0.2757	0.2185	0.0052	0.1565	2.3259	1.3435	0.1339
Frequency	2	0.0702	0.2973	0.4934	0.2685	0.2704	0.3268	0.0029
Voltage x Frequency	2	0.0137	2.1439	3.3609	0.1499	0.1922	0.0019	0.1590
Experimental Error	15	0.2637	1.4442	3.1174	0.6175	0.9304	0.5624	0.1105
Sampling Error (Triplicate)	84	0.0247	0.0248	0.0428	0.0753	0.1156	0.0922	0.0699

APPENDIX B

ANALYSIS OF VARIANCE FOR CHAPTER III

TABLE 1

ANALYSIS OF VARIANCE OF pH AND TEMPERATURE DECLINE AT 0, 2, 4, 6, 8 and 24 HR POSTMORTEM IN LONGISSIMUS DORSI AS AFFECTED BY ELECTRICAL STIMULATION AND MODE OF CHILLING

Source of Variation	d.f.	Mean Square	
		pH	Temperature
Total	143		
Block (Animal	11		
Treatment (Adj.)	3	1.3847***	89.0796***
Hour	5	3.4410**	450.3548***
Treatment x Hour	15	0.1170**	16.4665***
Error A (Block x Treatment)	9	0.0313	1.2109
Error B (Block x Hour)	55	0.0266	4.1662
Error C (Block x Treatment x Hour)	45	0.0087	0.4565

**, ***Significant at $P<0.01$ and 0.005, respectively.

TABLE 2

ANALYSIS OF VARIANCE OF SHORTENING (%) OF SEMITENDINOSUS MUSCLE AT 24 HR POSTMORTEM AS AFFECTED BY ELECTRICAL STIMULATION AND MODE OF CHILLING

Source of Variation	d.f.	Mean Square
Total	47	
Block (Animal)	11	
Treatment (Adj.)	3	209.2122
Stimulation	1	431.4453***
Chilling	1	193.7989***
Stimulation x Chilling	1	2.3926
Experimental Error	9	11.8164
Sampling Error (Duplicate)	24	2.6367

***Significant at P<.005.

TABLE 3

ANALYSIS OF VARIANCE OF SHEAR FORCE VALUES IN BOTH LONGISSIMUS DORSI (LD) AND SEMITENDINOSUS (ST) MUSCLES AT 48 HR POSTMORTEM AS AFFECTED BY ELECTRICAL STIMULATION AND MODE OF CHILLING

Source of Variation	LD Muscle		ST Muscle	
	d.f.	Mean Square	d.f.	Mean Square
Total	287		191	
Block (Animal	11		11	
Treatment (Adj.)	3	35.2690	3	14.6113
Stimulation	1	102.4482***	1	39.1170***
Chilling	1	2.9304	1	4.6818*
Stimulation x Chilling	1	0.4285	1	0.0351
Experimental Error	9	1.2698	9	0.8200
Sampling Error	264	0.6654	168	0.6292

*, ***Significant at P<.05 and 0.005, respectively.

TABLE 4

ANALYSIS OF VARIANCE OF COOKING LOSS (%) IN BOTH LONGISSIMUS DORSI (LD) AND SEMITENDINOSUS (ST) MUSCLES AT 24 HR POSTMORTEM AS AFFECTED BY ELECTRICAL STIMULATION AND MODE OF CHILLING

Source of Variation	d.f.	Mean Square	
		LD Muscle	ST Muscle
Total	47		
Block (Animal)	11		
Treatment (Adj.)	3	1.3415	1.1827
Stimulation	1	0.0028	0.3424
Chilling	1	3.7607	0.1128
Stimulation x Chilling	1	0.2610	3.0928
Experimental Error	9	2.9551	3.2278
Sampling Error	24	1.9337	0.9602

TABLE 5

ANALYSIS OF VARIANCE OF HUNTERLAB "L, a, and b" COLOR VALUES AND a/b COLOR RATIO IN LONGISSIMUS DORSI MUSCLE FROM DAY 1 TO DAY 4

Source of Variation	d.f.	Mean Square			
		L	a	b	a/b
Total	191				
Block	11				
Treatment (Adj.)	3	0.0425	1.6539	0.1166	0.0408
Day	3	101.4758***	2.8937	54.7256***	4.5159***
Treatment x Day	9	0.8013	0.4635	0.1467	0.0187
Error A (Block x Treatment)	9	0.7615	1.6060	0.2329	0.0402
Error B (Block x Day)	33	1.2345	1.9684	0.6995	0.0400
Error C (Block x Treatment x Day)	27	0.5021	0.4854	0.0803	0.0077
Error D (Chilling x Block x Treatment x Day)	96	0.3421	0.2489	0.0466	0.0059

***Significant at P<0.005.

TABLE 6

ANALYSIS OF VARIANCE OF SARCOPLASMIC (SP), MYOFIBRILLAR EXTRACTED WITH 0.3 M NaCl UNBUFFERED (MF_1), MYOFIBRILLAR EXTRACTED WITH 0.6 M Kl IN 0.1 M PHOSPHATE BUFFER (MF_2), TOTAL MYOFIBRILLAR (TMF), AND INTRACELLULAR PROTEINS (ICP) VALUES IN LONGISSIMUS DORSI MUSCLE AT 24 HR POSTMORTEM

Source of Variation	d.f.	Mean Square				
		SP	MF_1	MF_2	TMF	ICP
Total	71					
Block (Animal)	11					
Treatment (Adj.)	3	0.0561	0.1605	0.0387	0.1241	0.1123
Stimulation	1	0.0875	0.1541	0.1102	0.0036	0.1271
Chilling	1	0.0325	0.0169	0.0037	0.0048	0.6624
Stimulation x Chilling	1	0.0481	0.3104	0.0021	0.3640	0.1474
Experimental Error	9	0.1706	0.0723	0.0627	0.0717	0.2507
Sampling Error	48	0.0107	0.0179	0.0164	0.0218	0.0428

TABLE 7

ANALYSIS OF VARIANCE OF ACID-SOLUBLE PROTEIN (ASP), ACID-INSOLUBLE STROMAL PROTEIN (AIP), EXTRACELLULAR (EC), TOTAL PROTEIN (TP), AND THE SWELLING FACTOR (SF) OF STROMA VALUES IN LONGISSIMUS DORSI MUSCLE AT 24 HR POSTMORTEM

Source of Variation	d.f.	Mean Square				
		ASP	AIP	EC	TP	SF
Total	71					
Block (Animal)	11					
Treatment (Adj.)	3	0.0557	0.0135	0.0342	0.0861	0.0118
Stimulation	1	0.0990	0.0000	0.0990	0.0017	0.0042
Chilling	1	0.0005	0.0003	0.0000	0.0602	0.0300
Stimulation x Chilling	1	0.0675	0.0402	0.0035	0.1963	0.0011
Experimental Error	9	0.0364	0.4070	0.3062	0.0685	0.0076
Sampling Error	48	0.0115	0.0519	0.0487	0.0287	0.0057

VITA

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