EFFECTS OF ELECTRICAL STIMULATION ON TOTAL CALPAIN ACTIVITY AND QUALITY CHARACTERISTICS OF IRANIAN FAT-TAILED SHEEP CARCASSES

M. Abbasvali [1]*, S.S. Shekarforoush [1], M. Aminlari [2]

[1]Department of Food Hygiene and public health, School of Veterinary Medicine, Shiraz University, Shiraz 71345-1731, Iran
[2]Department of Biochemistry, School of Veterinary Medicine, Shiraz University, Shiraz 71345-1731, Iran
*Corresponding author (phone: +98 711 2286950; fax: +98 711 2286940; e-mail: maryamevali@yahoo.com)

Abstract

Electrical stimulation (ES) has been reported to improve meat quality. In this study, the combination effects of electrical stimulation with different voltage/duration and early postmortem (PM) rigor temperature (4 °C and 25 °C until 4 h PM) on PM changes and meat quality of 24 male Iranian fat-tailed sheep carcasses were evaluated, including adenosine triphosphate (ATP) content, total calpain activity, water holding capacity (WHC) and muscle color of *longissimus dorsi* (LD) muscle. Each carcass was subjected to one of the eight treatments. In two experimental groups (T1 and T2) no stimulation were subjected (controls) and early PM temperature conditioning 4 °C and 25 °C until 4 h PM, were respectively used. Furthermore, six groups (T3, T4, T5, T6, T7 and T8) were stimulated five minutes after bleeding with different voltages (100 and 150V), duration (30 and 60 sec) and fixed frequency (50 Hz), and rigor temperature (4 °C and 25 °C until 4 h PM). Each carcass was compared to its corresponding non-ES control group. ATP content was measured at 1, 3, 6, 12 and 24 h PM. Total calpain activity was determined at 1, 3 and 6 h PM. Also, on 1, 2, 3, 7 and 14 days PM samples were taken from the LD muscle of each carcass and used for analyses of WHC and muscle color. The results showed significant reduction in ATP content ($P < 0.05$). There was a significantly ($P < 0.05$) gradually decline in total calpain activity after 6 h PM. Muscles from ES carcasses had significantly ($P < 0.05$) lower WHC than those from non stimulated ones. No significant difference found for the mean muscle color values from all treatments ($P > 0.05$) and color values (*L, *a**, *b**) were not affected by ES. The results of this experiment indicated that electrical stimulation with higher voltage/duration (150v/60sec) and early rigor temperature at 25˚C until 4 h PM was more effective on acceleration of ATP depletion, early activation of calpain system and reduction of total calpain activity later on, than other treatments, Also showed lowest WHC. Electrical stimulation had no influence on meat color in any treatments.**

Key words: **Electrical stimulation, Fat-tailed sheep, Meat color, Total calpain activity**

I. INTRODUCTION

Postmortem electrical stimulation has been shown in previous studies (Polidori, Lee, Kauffman & Marsh, 1999; Toohey, Hopkins, Stanley & Nielsen, 2008; Kadim, Mahgoub, Al-Marzooqi, Khalaf, Al-Sinawi & Al-Amri, 2009) to improve many quality factors in lamb meat.

Fat- tailed sheep is one of the most important food animals in Iran and its meat is very popular for consumers. The carcass quality of these sheep is quite good, with most of the fat concentrated in the tail area. Data on postmortem muscle metabolism and development of meat quality of Iranian fat-tailed sheep are scanty.

The objectives of this experiment were to determine the effects of different voltage/duration of electrical stimulation with early rigor temperature 4 and 25 °C until 4 h PM on total calpain activity and quality characteristics of Iranian fat-tailed sheep carcasses.

II. MATERIALS AND METHODS

A. Animals and experimental design

A total of 24 male Iranian fat tailed sheep were used for this experiment. In the first experimental group (T1) carcasses without any ES were chilled at 4 °C for 14 days PM, as control for treatment groups of T3, T4, T6 and T7. Carcasses in the second group (T2) without any ES, were kept at 25 °C for 4 h, and then placed in a chiller at 4 °C for 14 days. They were used as control group for treatment groups of T5 and T8. Five minutes after bleeding, carcasses in the treatment groups of T3, T4, T6 and T7 were stimulated with 100v 30s, 100v 60s, 150v 30s and 150v 60s with fixed frequency of 50 Hz, respectively and were chilled at 4 °C for 14 days. Carcasses in the treatment groups of T5 and T8 were stimulated 100v 60s and 150v 60s, respectively and were kept at 25 °C for 4 h, and then placed in a chiller at 4 °C for 14 days. Samples were collected from both treated and control groups from M. *longissimus dorsi* at different times PM.

B. ATP analysis

Samples for adenosine triphosphate (ATP) analysis were removed at 1, 3, 6, 12 and 24 h PM. Samples were immediately frozen and stored at -70˚C until assayed, using high performance liquid chromatographic method described by Watanabe, Tsuneishi and Takimoto (1989).

C. Calpain activity assay

Total calpain activity was determined by fluorometric method. Samples removed for quantification of calpain activities (1, 3 and 6 h PM) were preserved at -70 °C until utilization. Total calpain activity was measured using a calpain activity assay kit (Calbiochem, USA) according to the manufacturer's instructions. The results were displayed in a relative fluorescence unit (RFU).

D. Water holding capacity

The water holding capacity (WHC) of the meat samples were estimated on 1,2,3,7 and 14 day aged samples using the method described by Grau and Hamm (1950) with slight modification. Briefly, 0.3 g meat samples were collected from identical places of the control and treated samples. The samples were placed on the Whatman filter paper No. 2 and pressed at a constant pressure (1kg) for 20 min. During the stipulated time, water released gives an impression on the filter paper. The impression on filter paper was measured by planimeter and changes in drip over times postmortem were shown with area of the impression (in square cm), that provides an index of the WHC to which it is inversely related.

E. Color measurement

Meat color was measured on 1,2,3,7 and 14 day aged samples. Color values L*, a* and b* were determined with a simple digital imaging method described by Yam and Papadakis (2004), with slight modification. Samples were removed by cutting in a transverse direction across the LD muscle to expose a fresh surface to air and were allowed to bloom for 30 min at room temperature prior to color measurement. The images used in this study were taken with Canon PowerShot G9 color digital camera with 12 Mega Pixels of resolution and were analyzed using *Adobe Photoshop 8.0* to obtain L*a*b* color values. Color was evaluated by obtaining three measurements per sample, which were then averaged to obtain a mean value for L*, a*, and b* for each muscle sample.

F. Statistical analyses

The results were evaluated by one-way analysis of variance technique and Duncan's multiple-range test. All statistical analyses were performed by SPSS software, version 11.

III. Results and discussion

Significantly ($P<0.05$) more ATP was found in control samples than in electrically stimulated samples at times post stimulation (Figure 1). The accelerated depletion of ATP in stimulated muscles is in agreement with other experiments published (Will, Henrickson, Morrison & Odell, 1979; Polidori et al., 1999).

Total calpain activity declined significantly ($P < 0.05$) in the first 6 h PM in stimulated muscles (Figure 2). The accelerated glycolysis by stimulation was reported to cause earlier activation of calpain and/or calpastatin activity (Rhee & Kim, 2001). This leads to the earlier decrease of enzyme activity, possibly due to autolysis (inactivation by itself and calpastatin) and proteolysis (tenderisation) (Hwang &Thompson, 2001a; Hwang &Thompson, 2001b). According to Hwang and Thompson (2001a), early activation of the calpain system appears to be an important mechanism by which electrical stimulation improves meat tenderness in general or at least early postmortem.

The WHC of the LD of electrically stimulated muscles were found to be significantly ($P < 0.05$) less than those of the controls and dripping increased so WHC decreased during ageing (Figure 3). In agreement with the present results, Den Hertog-Meischke, Smulders, Van Logtestijin and Van Kanapen, (1997) found that filter paper wetness was significantly higher for stimulated bovine muscles than for non-stimulated ones. Kadim et al. (2009) reported the increase in myofibrillar expressed juice of electrically-stimulated muscles. Rosenvold et al. (2008) reported, at a constant ageing temperature after rigor mortis, drip increased as meat tenderised. It was proposed that the drip was released from the cytoskeletal proteins as they degrade during the ageing process, their findings supported our data. In the current study, the differences between samples from stimulated and non-stimulated muscle are probably a result of shrinkage of myofibrils due to pH fall postmortem and denaturation of protein due to low pH and high temperature (Strydom, Frylinck & Smith, 2005).

There were no significantly differences ($p > 0.05$) between electrical stimulated and non stimulated samples for L*, a*, and b* values (Table 1). Similar results were found in studies by Channon, Baud and Walker, (2005) and Toohey et al. (2008) where they were determined that the use of electrical stimulation had no effect on the meat color of lamb. Bond, Can and Warner, (2004) found that electrical stimulation of sheep carcasses did not improve muscle color lightness.

IV. Conclusion

Combination of higher voltage/duration of electrical stimulation (150v, 60s) and 25 °C conditioning until 4 h PM showed greater effects on acceleration of postmortem ATP depletion, early activation of calpain and reduction of total calpain activity later on. Also, showed lowest WHC and had no influence on meat color.

References

Bond, J.J., Can, L.A., &Warner, R.D. (2004). The effect of exercise stress, adrenaline injection and electrical stimulation on changes in quality attributes and proteins in semimbranosus muscle of lamb. Meat Science, 68, 469–477.
Channon, H. A., Baud, S. R., & Walker, P. J. (2005). Modified atmosphere packaging improves retail display life of lamb cuts with variation between loin and Knuckle. Australian Journal of Experimental Agriculture, 45, 585–592.
Den Hertog-Meischke, M.J.A., Smulders, F.J.M., Van Logtestijin, J.G., & Van Kanapen, F. (1997). The effect of electrical stimulation on the waterholding capacity and protein denaturation of two bovine muscles. Journal of Animal Science, 75, 118–124.
Grau, R., & Hamm, R. (1950). Cited by Price, J. F., & Schweigert, B. S.,In The science of meat and meat products (2nd ed., p. 206).Sanfrancisco: WH Freeman and Company.
Hwang, I. H., & Thompson, J. M. (2001a). The effect of time and type of electrical stimulation on the calpain system and meat tenderness in beef longissimus dorsi muscle. Meat Science, 58, 135–144.
Hwang, I. H., & Thompson, J. M. (2001b). The interaction between pH and temperature decline early postmortem on the calpain system and objective tenderness in electrically stimulated beef longissimus dorsi muscle. Meat Science, 58, 167–174.
Kadim, I.T., Mahgoub, O., Al-Marzooqi, W., Khalaf, S., Al-Sinawi, S.S.H., & Al-Amri, I.S. (2009). Effects of transportation during the hot season and low voltage electrical stimulation on histochemical and meat quality characteristics of sheep longissimus muscle. Livestock Science, 126, 154–161.
Polidori, P., Lee, S., Kauffman, R. G., & Marsh, B. B. (1999). Low voltage electrical stimulation of lamb carcases: Effects on meat quality. Meat Science, 53, 179–182.
Rhee, M. S., & Kim, B. C. (2001). Effect of low voltage electrical stimulation and temperature conditioning on postmortem changes in glycolysis and calpains activities of Korean native cattle (hanwoo). Meat Science, 58, 231–237.
Rosenvold, K., North, M., Devine, C., Micklander, E., Hansen, P., Dobbie, P., & wells, R. (2008). The protective effect of electrical stimulation and wrapping on beef tenderness at high pre rigor temperatures. Meat Science, 79, 299-306
Strydom, P. E., Frylinck, L., & Smith, M. F. (2005). Should electrical stimulation be applied when cold shortening is not a risk? Meat Science, 70, 733–742.
Toohey, E.S., Hopkins, D.L., Stanley, D.F., & Nielsen, S.G. (2008). The impact of new generation pre-dressing medium-voltage electrical stimulation on tenderness and colour stability in lamb meat. Meat Science, 79, 683–691.
Watanabe, A., Tsuneishi, E., & Takimoto, Y. (1989). Analysis of ATP and Its Breakdown Products in Beef by Reversed- Phase HPLC. Journal of Food Science, 54 (5), 1169- 1172.
Will, P. A., Henrickson, R. L., Morrison, R. D., & Odell, G. V. (1979). Effect of electrical stimulation on ATP depletion and sarcomere length in delay-chilled bovine muscle. Journal of Food Science, 44, 1646-1648.
Yam, K.L., & Papadakis, S.E. (2004). A simple digital imaging method for measuring and analyzing color of food surfaces. Journal of Food Engineering, 61, 137–142.

Table1. Effects of electrical stimulation and rigor temperature (4 °C and 25 °C until 4 h of postmortem) on meat color values of the muscle samples of Iranian fat tailed sheep during postmortem storage at 4 °C.

Color value	Treatment groups	Days of postmortem				
		1	2	3	7	14
L*	T1	43.67 ± 1.67	46.33 ± 2.19	47.00 ± 1.41	47.00 ± 1.00	44.00 ± 1.53
	T2	41.67 ± 1.45	44.33 ± 0.33	46.00 ± 0.58	48.33 ± 0.88	48.33 ± 1.67
	T3	42.00 ± 0.58	43.67 ± 1.20	43.67 ± 1.23	44.67 ± 1.67	44.33 ± 0.33
	T4	42.33 ± 2.33	44.00 ± 1.00	44.00 ± 0.52	45.33 ± 1.20	46.33 ± 1.45
	T5	41.33 ± 1.20	45.33 ± 1.45	45.00 ± 0.58	48.00 ± 1.00	46.67 ± 1.20
	T6	42.67 ± 0.67	46.00 ± 1.00	43.17 ± 1.19	46.00 ± 1.00	45.67 ± 1.45
	T7	42.33 ± 0.88	44.67 ± 1.20	43.17 ± 0.75	47.67 ± 1.67	45.67 ± 0.88
	T8	45.33 ± 1.45	47.00 ± 1.73	46.00 ± 1.00	47.67 ± 1.20	45.00 ± 2.08
a*	T1	28.00 ± 0.58	26.67 ± 0.67	26.75 ± 0.48	28.00 ± 1.53	27.67 ± 1.45
	T2	28.00 ± 1.15	26.67 ±0.33	26.67 ± 0.67	28.00 ± 0.58	29.00 ± 0.00
	T3	28.00 ± 2.00	25.33 ± 1.33	25.00 ± 1.10	26.33 ± 2.33	27.47 ± 1.77
	T4	28.00 ± 2.08	25.00 ±1.00	25.50 ± 0.96	28.00 ± 3.79	27.67 ± 2.33
	T5	30.67 ± 1.45	25.33 ± 0.33	26.00 ± 1.53	27.33 ± 0.33	27.67 ± 1.20
	T6	28.33 ± 0.88	25.67 ± 1.20	25.00 ±0.82	27.00 ± 2.52	27.33 ± 1.86
	T7	25.67 ± 0.33	26.00 ± 1.15	25.50 ±0.76	28.33 ± 3.38	28.67 ± 0.67
	T8	31.33 ± 0.88	27.67 ± 0.67	26.67 ± 1.45	29.04 ± 1.33	28.67 ± 0.33
b*	T1	14.67 ± 1.20	14.00 ± 1.53	13.00 ± 0.82	12.67 ± 1.45	13.33 ± 1.67
	T2	15.33 ± 0.33	13.67 ± 0.33	13.00 ± 0.58	14.33 ± 0.88	13.67 ± 0.67
	T3	13.00 ± 0.58	14.00 ±0.00	13.33 ± 0.21	13.00 ± 1.00	14.27 ± 0.87
	T4	12.67 ± 0.33	13.67 ±0.33	13.50 ± 0.72	14.33 ± 1.45	14.67 ± 0.33
	T5	14.33 ± 0.88	15.00 ±1.15	14.67 ± 0.33	14.67 ± 2.03	15.00 ± 0.58
	T6	15.00 ± 0.58	13.33 ±1.76	13.78 ± 0.58	14.67 ± 0.88	15.00 ± 0.58
	T7	12.67 ± 0.33	13.00 ±1.15	13.00 ± 0.68	13.67 ± 0.33	14.33 ± 0.33
	T8	15.67 ± 0.88	17.00 ±1.00	14.33 ± 1.67	16.67 ±1.86	13.67 ± 1.45

T1: without any electrical stimulation, chilled at 4 °C; T2: without any electrical stimulation, kept at 25 °C for 4 h, and then at 4 °C; T3: stimulated with 100V, 30 sec., chilled at 4 °C; T4: stimulated with 100V, 60 sec., chilled at 4 °C; T5: stimulated with 100V, 60 sec., kept at 25 °C for 4 h, and then at 4 °C; T6: stimulated with 150V, 30 sec., chilled at 4 °C; T7: stimulated with 150V, 60 sec., chilled at 4 °C; T8: stimulated with 150V, 60 sec., kept at 25 °C for 4 h, and then at 4 °C.

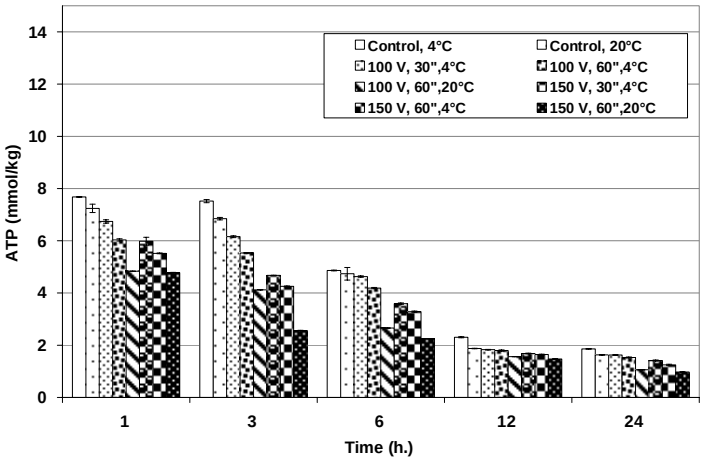

Figure1. Effects of electrical stimulation and rigor temperature (4 °C and 25 °C until 4 h of postmortem) on ATP depletion of *longissimus dorsi* muscle of Iranian fat tailed sheep during postmortem storage at 4 °C.

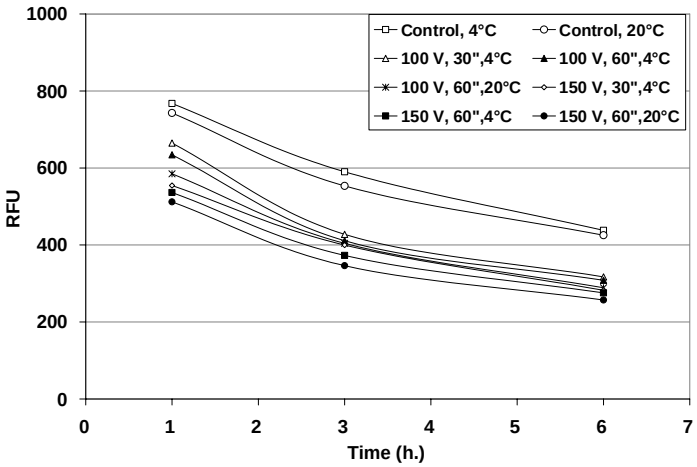

Figure2. Effects of electrical stimulation and rigor temperature (4 °C and 25 °C until 4 h of postmortem) on total calpain activity of *longissimus dorsi* muscle of Iranian fat tailed sheep during postmortem storage at 4 °C.

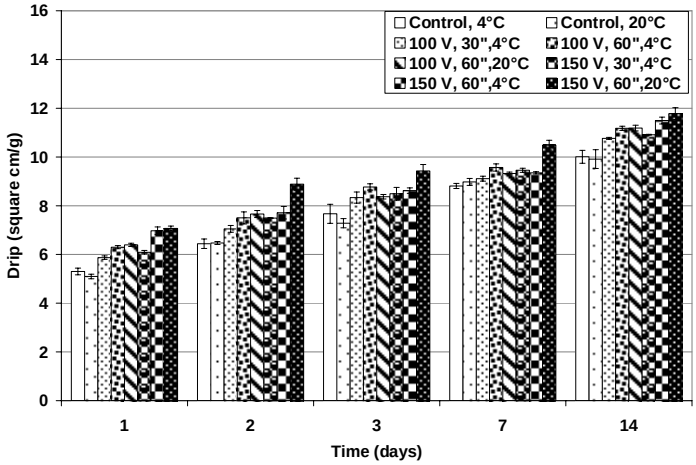

Figure3. Effects of electrical stimulation and rigor temperature (4 °C and 25 °C until 4 h of postmortem) on changes in drip of *longissimus dorsi* muscle of Iranian fat tailed sheep during postmortem storage at 4 °C.