

The effects of low-voltage electrical stimulation and freezing on tenderisation, enzyme activities, drip losses and cooking losses of lamb

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SUMMARY

The effects of low voltage electrical stimulation and freezing on texture, enzyme levels, drip loss and cooking loss were studied in 24 wether lamb carcasses averaging 17 kg. Immediately after slaughtering, 12 carcasses were electrically stimulated for sixty seconds using 85 V at 14 Hz. The 24 carcasses were split and held at 10°C. After 6 hours 1/3 of the sides were frozen at -20°C with an air speed of 1-2 m/s. The remaining sides were held for 24 hours at 10°C, butchered, the loins removed, and one half frozen at -20°C and the other half chilled at 2°C. After a week's frozen storage at -20°C the frozen loins were thawed at 7°C for 24 hours. Fresh loins and thawed loins were stored at 2°C for up to 8 days and tested for texture, enzyme levels, drip loss and cooking loss.

Low voltage electrical stimulation or freezing did not affect the rate of tenderisation during chill storage. Meat frozen at 6 hours was tougher and meat frozen at 24 hours more tender than the control fresh meat. Electrical stimulation and freezing lowered the activity of calpain I and freezing lowered the level of the calpain inhibitor. Freezing increased the levels of cathepsins B and L in sarcoplasmic fluid.

Lamb held for 24 hours before freezing and thawed slowly in 24 hours was found to be acceptably tender upon thawing even without electrical stimulation.

Key words: calpain, calpastatin, cathepsin, freezing, electrical stimulation, lamb, tenderisation.

YFIRLIT

Áhrif rafmagnsörvunar og frystingar á meyrun, hvatastarfsemi og vökvatap lambakjöts

Áhrif lágsþennu-rafmagnsörvunar og frystingar á seigju, hvatamagn og vökvatap var athugað í 24 lambsföllum, sem vógu að meðaltali 17 kg. Tólf þeirra voru rafmagnsörvuð í 60 sek. með lágsþennu (85 V, 14 Hz), strax eftir aflífun. Skrokkarnir voru klofnir að endilöngu og látirir hanga við 10°C. Eftir 6 klst. var þriðjungur klofinna skrokkanna frystur við -20°C og lofthraða 1-2 m/s. Sólarhring eftir slátrun voru hryggirnir fjarlægðir úr skrokkunum sem eftir voru. Helmingur þeirra var frystur við -20°C en hinn helmingurinn var settur í kæli við 2°C. Frosna kjötið var geymt við -20°C í viku, en þá þitt í kæli við 7°C í sólarhring. Eftir þessa meðferð var allt kjötið geymt í kæli við 2°C í átta daga og rannsakað með tilliti til seigju, hvatamagns og vökvataps.

Lágsþennu-rafmagnsörvun og frysting hafði ekki áhrif á hraða meyrunar. Kjöt fryst 6 klst. eftir

slátrun var seigara og kjöt fryst 24 klst. eftir slátrun meyrara en kjöt sem ekki var fryst. Rafmagnsörvun og frysting minnkaði kalpein I í kjötinu og frysting minnkaði kalpastatín. Frysting jók katepsín B og L í frumvökva.

Lambakjöt, sem látið var hanga í sólarhring fyrir frystingu við 10°C, var viðunandi meyrir eftir uppþíðingu þó að það hefði ekki verið rafmagnsörvað.

INTRODUCTION

In most countries the supply of lamb meat is not evenly distributed throughout the year because sheep are seasonal breeders and the lambs are usually grown to slaughter weight on grass which grows only during the warmer months. The latter is especially true in northern countries with a limited grazing season, which in Iceland, for example, can be as short as three to four months, and to a lesser degree in countries with a longer grazing period as the U.K. In the U.K. the average annual consumption of lamb is less than 4 kg per capita (MLC, 1988) but in Iceland the average annual consumption of lamb is over 30 kg per capita (IAPRS, 1990) and lamb is only slaughtered during a 6 week period in September and October. This compact production of lamb makes it necessary to preserve large quantities of meat by chilling or freezing. The freezing of lamb is usually done within 24 hours of slaughter because of the high throughput of the slaughterhouses and the limited amount of chiller space.

Early freezing may induce muscle contraction and the meat will become extremely tough upon cooking. This phenomenon is called cold shortening (Locker and Hagyard, 1963; Bendall, 1972). If meat is frozen before onset of rigor and rapidly thawed there will again be an extreme shortening together with increased drip loss. This is called thaw shortening. However, if this meat is thawed slowly, thaw shortening is avoided (Marsh and Thompson, 1958) and tender meat obtained (Dransfield, 1974).

Shortening may be reduced by delaying chilling so that the temperature of the muscles will not reach 10°C within 10 hours of slaughter in beef and lamb (Bendall, 1972) when the pH will have fallen below 6.2; by

suspending lambs so they will go into rigor in a standing posture (Davey and Gilbert, 1973, 1974) or by employing electrical stimulation to increase the rate of early post-mortem glycolysis (Taylor, 1981).

The production of high quality meat necessitates a period of conditioning. To achieve 80% tenderisation through conditioning lamb meat has to be stored at chill temperatures, 1–4°C, for about 7 days (Dransfield, 1986). The enzymes that are thought to be mainly responsible for the conditioning of meat, the calcium-activated neutral proteinases, calpains I and II, and the lysosomal cathepsins, have been isolated from different species (Etherington *et al.*, 1987). Their effectiveness in tenderisation may be increased by electrical stimulation and freezing. Electrical stimulation may disrupt the lysosomal membranes releasing cathepsins (Dutson *et al.*, 1980). Freezing will release cathepsins, which are relatively stable, from the lysosomes (Davies, 1975) and very rapid freezing reduces the inhibitor activity of calpastatin, a natural inhibitor of calpain (Koochmarai, 1990a). However, it is not known how stable calpains and calpastatin are under normal commercial freezing rates.

MATERIALS AND METHODS

Experimental plan

The quality of nonstimulated (NES) and electrically stimulated (ES) lamb frozen at 6 hours (F6) or 24 hours (F24) after slaughter and later thawed for chill storage and chilled lamb (Ch) was measured throughout chilled storage for up to 8 days.

Animals and slaughter

Twenty-four Texel × Mule wether lambs were

killed in groups of six at 2 weeks intervals by electrical stunning and dressed in a conventional manner. A further four lambs were slaughtered to complete the enzyme studies. The carcasses averaged 17 kg.

Experimental procedures

The twenty four carcasses were treated in a balanced design to compare the three treatment groups with and without electrical stimulation.

Low-voltage electrical stimulation. Low-voltage electrical stimulation was applied to the carcasses immediately after sticking and during bleedout. Stainless steel hook electrodes were placed in the musculature of each hind leg close to the Achilles tendon and two steel clips were placed on the upper and the lower lips on the head of the carcass. Stimulation at 85 V, 14 Hz was applied for sixty-four seconds.

Cooling. The carcasses were moved into a holding area at 10°C forty minutes after stunning. The sides remained in the holding area until the time for chilling or freezing.

Those sides which were frozen at 6 hours after slaughter were hung by the hocks in a freezing room at -20°C with an air velocity of 1-2 m/s.

Butchery. All the sides were butchered 24 hours after slaughter and the loins removed. The non-frozen sides were butchered conventionally whereas a bandsaw was employed to butcher the frozen loins. All the loins were then vacuum packed.

Frozen storage. The loins which were frozen at 24 hours after slaughter were put on racks and frozen at -20°C with an air velocity of 1-2 m/s. All the loins were then stored in their vacuum packs under these conditions for 7 days.

Thawing. The frozen loins were thawed on racks at 7°C for 24 hours.

Chill storage. The *M. longissimus dorsi* (L.D.) from all the loins were removed and then divided into three samples and vacuum packed

individually for the different treatments. One sample was tested immediately and the other two were stored in vacuum packs at 2°C for 2 days and 7 days, respectively.

Cooking. The samples were cooked in a water bath at 80°C to a core temperature of 78°C and then held for a further 10 min, taking approximately 40 min to complete the cooking. The samples were then chilled in cold water and stored overnight in air at 1°C.

Measurements

Temperature. Temperatures of the deep leg were monitored continuously and recorded at 10 min intervals using a Squirrel recorder from the time the carcasses entered the holding area and during freezing and thawing.

pH. The pH was measured by excising a sample of L.D. weighing approximately 1 g. The excised samples were placed in 10 ml of 5 mM Iodoacetate and 150 mM KCl buffer, pH 7, homogenised immediately to prevent any further fall in pH and stored at 1°C. The pH of the samples was measured at a convenient time using a standard glass electrode pH meter.

Drip loss. The drip loss of the samples was measured by weighing the samples before and after chill storage and was expressed as a percentage of the original weight.

Enzymes. The activity of calpain I and II, calpastatin and cathepsins B and L were measured in L.D. muscle extracts and sarcoplasmic fluid.

Preparation of calpains. Calpains were prepared according to the method of Etherington *et al.* (1987). Muscle was trimmed of excess fat and connective tissue and a 5 g sample was homogenised on ice with 45 ml 50 mM Tris/HCl + 3 mM EDTA and 150 mM pepstatin, pH 7.5, using a Polytron homogenizer. The pH was readjusted to 7.5 with 1 M NaOH. The homogenate was stirred at 4°C for 1 hour and then centrifuged at 10 000 g for 10 min. The supernatant was filtered

through a glass fibre filter disk to remove fatty material and then adjusted to 0.5 M NaCl and 2 μ M Leupeptin (from 10 mM stock in DMSO). Ten milliliters of supernatant were then loaded onto a 2 ml Phenyl-Sepharose minicolumn (Biorad) previously equilibrated with Tris/HCl + 3 mM EDTA + 150 nM Pepstatin + 0.5 M NaCl, pH 7.5. The unadsorbed proteins, including the inhibitor calpastatin, were eluted with 2x4 ml of the same buffer and the calpains were eluted with 2x2 ml of 50% (v/v) ethylene glycol in 50 mM Tris/HCl + 3 mM EDTA, pH 7.5.

Separation of calpain I and calpain II. Calpain I and calpain II were separated according to the method of Etherington *et al.* (1987) using an FPLC fitted with a Mono Q, anion exchange column, Fraction collector, Frac-100, and a flat-bed recorder (Pharmacia, Uppsala, Sweden). The calpains, free of calpastatin, were adsorbed to the Mono Q column which had been equilibrated with 20 mM Bis-Tris-Propane buffer, pH 7.2 and eluted with a linear gradient of 0–60% 20 mM Bis-Tris-Propane + 1M NaCl buffer, pH 7.2. Fractions of 1 ml each were collected and assayed for calpain activity (Figure 1a). A baseline of absorbance was selected "by eye" and the calpain activities of 3 fractions combined.

Measurement of calpain activity. Calpain activity was assayed by the method of Etherington *et al.* (1987) using casein as a substrate. Twenty microliters of enzyme + 180 μ l 0.8% Hammarsten casein in 100 mM Tris/HCl + 5 mM CaCl₂ + 2 mM dithiothreitol, pH 7.5, were incubated for 20 min at 30°C and the reaction stopped with 200 μ l 5% (w/v) trichloroacetic acid. The suspension was centrifuged at 10 000 g for 10 min to remove insoluble protein and 0.2 ml of the supernatant were analysed for soluble peptides by diluting with 0.6 ml water and adding 0.2 ml Biorad protein assay reagent. The optical density was measured after 5 min at 595 nm in a Pye Unicam, SP500, series 2, ultraviolet and visible spectrophotometer. The activity of

calpain was expressed as μ g casein hydrolysed per min per g muscle.

Separation of calpastatin. Calpastatin was separated from calpains by the method of Etherington *et al.* (1987). The method is the same as employed for the preparation of calpains. The unadsorbed proteins in the 10 ml of supernatant, including calpastatin, were eluted first from the Phenyl-Sepharose column and the remaining unadsorbed protein was washed out with 2x4 ml 50 mM Tris/HCl + 3 mM EDTA + 0.5 M NaCl buffer, making a total volume of 18 ml.

Calpastatin assay. Calpastatin activity was assayed by mixing 20 μ l aliquots of a calpain solution of known specific activity with increasing amounts of calpastatin. The inhibitor in the assay tubes was increased in 10 μ l

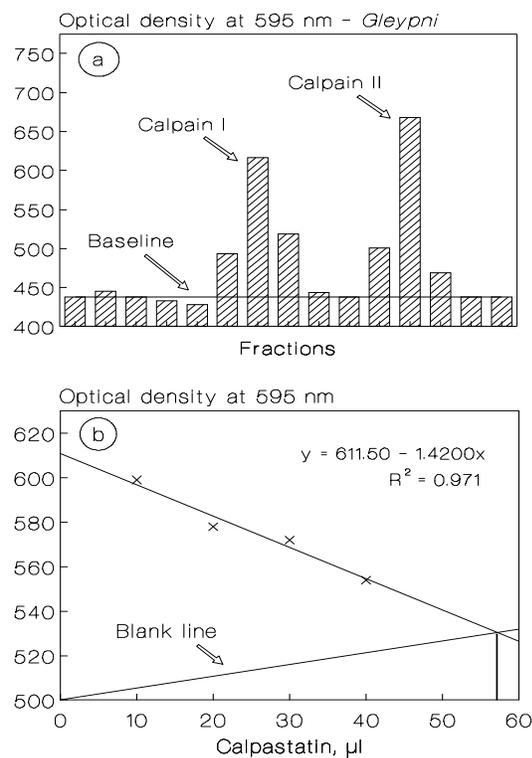


Figure 1. Activities of calpain I and II (a) and calpastatin at 24 hours (b).

1. mynd. Hvatavirgni kalpeins I og II (a) og kalpastatíns 24 klst. eftir slátrun.

increments. The volume of the mixture was adjusted with Tris/HCl buffer, pH 7.5, to a volume of 110 μ l and 90 μ l of casein (1.6% w/v) in 100 mM Tris/HCl buffer, pH 7.5, containing 10 mM CaCl₂ and 4 mM dithiothreitol was added and the mixture incubated at 30°C for 20 min. The reaction was stopped with 200 μ l 5% (w/v) trichloroacetic acid. After centrifugation the supernatant was analysed for protein using the Bio-Rad protein assay reagent.

Calculation of calpastatin activity. To calculate the calpastatin activity the OD₅₉₅ values were plotted as the example in Figure 1b. First a baseline was plotted from the activity of the calpastatin fraction incubated without enzyme. The values of increasing amounts of calpastatin incubated with a calpain of known activity were then plotted and the amount of calpastatin necessary to inhibit a 20 μ l amount of enzyme read from where the straight line plot intersected the baseline. One unit of inhibitor is defined as the amount needed to totally inhibit the activity of 1 unit of calpain.

Preparation of muscle extract for cathepsins.

The muscle extracts were prepared according to the method of Etherington *et al.* (1987). Five grams of muscle mince were homogenised in 45 ml of ice cold 50 mM Sodium citrate buffer, pH 5.0, containing 1 mM EDTA and 2% (v/v) Triton X-100. The extract was stirred for 1 hour at 4°C and centrifuged at 10 000 g for 10 min. The supernatant was then filtered through a glass fibre filter to remove fat.

Collection of sarcoplasmic fluid for cathepsins.

The muscle samples were minced and approximately 10 g from each sample was put into separate 14 ml centrifugation tubes and centrifuged at 14 000 g for 20 min and the supernatant pipetted off.

Assay of cathepsin B and L activity. The activity of cathepsin B and L was estimated fluorimetrically by the method of Etherington *et al.* (1987) using 10 μ M N-CBZ-L-phenyl-

alanyl-L-arginine-7-amino-4-methylcoumarin (Z-Phe-Arg-NHMeC) as substrate in a reaction volume of 3.2 ml at 37°C. The released 7-amino-4-methylcoumarin was monitored continuously using a flat bed recorder attached to a Perkin Elmer 3000 fluorimeter which was set at a wavelength of 340 nm for the excitation beam and 460 nm for the emission beam. The slit widths were set at 5 for both the excitation and the emission.

Calculation of activity of cathepsins B and L.

One unit of cathepsin activity was defined as the hydrolysis of 1 μ M of substrate per minute. To calculate the activity of cathepsins B and L free in sarcoplasmic fluid, the supernatant volume, was estimated as 0.75 ml of sarcoplasmic fluid being equivalent to 1 g of muscle sample. The free cathepsin B and L activity in sarcoplasmic fluid was calculated as a percentage of the total cathepsin B and L activity in muscle.

Cooking loss. The cooking loss was calculated by weighing the muscle samples before and after cooking and the cooking loss was expressed as a percentage of the raw weight.

Texture. Tenderness of the chilled samples was measured using an Instron TM-SM fitted with Volodkevich jaws, 50 kg load cell, crosshead speed 2.5 cm/min and chart speed 10 cm/min. Each cooked sample was cut into 8 blocks of 1x1 cm cross sectional area with the fibres running longitudinally and sheared perpendicular to the fibre axis. The shear force was calculated from the force/time curve by measuring the height of the first peak. The shear force was expressed as kg force calculated from an average of eight measurements of each sample.

Statistical analysis. The data recorded was analysed by analysis of variance, ANOVA, using the computer programme Minitab release 7.2, Minitab Inc. The programme calculated the means and the significance of any differences between the means and the significance of interaction between the treat-

ments. The Maximum Likelihood Programme, MLP, Rothamstead Experimental Station, was used to study the decrease in shear force and calpain I activity with time. The programme fitted an exponential decay curve to the data, fitted the individual curves to a common curve, compared the curves and calculated a rate constant. The data was analysed by ANOVA by the programme and the significance of differences calculated.

RESULTS

For clarity of comparisons thawed meat at 1 day of chill storage refers to immediately upon thawing, i.e. chill storage for thawed meat refers to storage in the thawed state.

Chilling

The mean temperature in the deep leg of 3 carcasses cooled at 10°C is shown in Figure 2a. Chilling commenced at 1 hour after slaughter at which time the mean temperature in the deep leg was 38.5°C. At 6 hours after slaughter the temperature in the deep leg was 17°C and at 24 hours the mean temperature in the deep leg had fallen to 10.5°C.

The mean temperature in the deep leg of 5 carcasses frozen at 6 hours after slaughter at -20°C, airspeed 1-2 m/s is shown in Figure 2b. The temperature reached -1°C, 10.5 hours after slaughter, i.e. 4.5 hours after freezing commenced, and the meat was frozen 12.5 hours after slaughter. The temperature had fallen to -20°C 18 hours after slaughter. The freezing time from 0° to -5°C was approximately 6 hours.

The temperature in the L.D. during thawing of 3 loins at 7°C is shown in Figure 2c. The thawing from -2°C to -1°C took approximately 11 hours and the mean temperature of the L.D. at the end of the thawing period of 24 hours was 0.5°C.

pH

The mean pH values in the L.D. are shown in Table 1. The mean pH value at 1 hour after slaughter was 6.72 in the nonstimulated

carcasses and 6.15 in those which had been electrically stimulated. The lowering of pH by the electrical stimulation at 1 hour after slaughter was highly significant. At 6 hours after slaughter the mean pH values of the nonstimulated carcasses was 6.29 compared

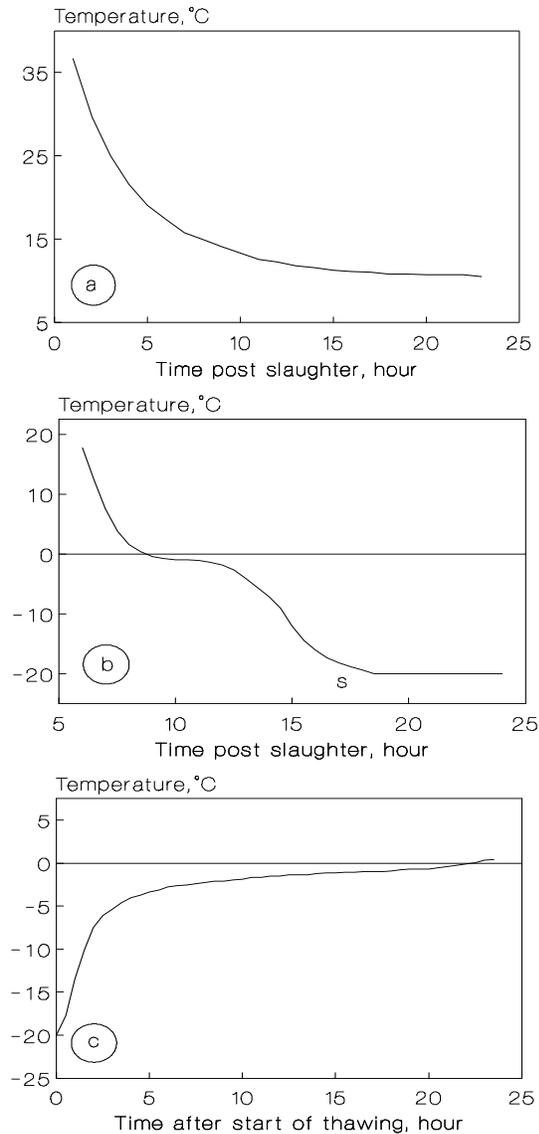


Figure 2. Rate of chilling at 10°C (a), rate of freezing at -20°C after 6 hours chilling (b) and rate of thawing at -7°C (c).

2. mynd. Kæling við 10°C (a), frýsting við -20°C eftir 6 klst. kælingu (b) og þíðing við -7°C (c).

Table 1. Mean pH values, standard deviation (SD) and significance of the differences (P) between stimulated (ES) and nonstimulated (NES.) carcasses at 1 hour, 6 hours and 24 hours after slaughter.

1. tafla. Meðalsýrustig, staðalfrávik og marktekt mismunar á raförvuðu (ES) og óörvuðu (NES) kjöti 1, 6 og 24 klst. eftir slátnun.

	NES	ES	P
1 hour	6.72 (0.1)	6.15 (0.1)	***
6 hours	6.29 (0.2)	5.75 (0.1)	***
Upon thawing	5.71 (0.1)	5.73 (0.1)	NS
24 hours	5.60 (0.1)	5.62 (0.1)	NS

with 5.75 for those that had been stimulated. At 24 hours after slaughter, the mean pH value of the stimulated carcasses was the same as that of the nonstimulated ones and averaged 5.61.

Freezing nonstimulated sides at 6 hours after slaughter, storing them for 7 days and then thawing in 24 hours reduced the pH to 5.71 whereas freezing and thawing stimulated sides did not affect the pH of the L.D.

Drip loss

The mean drip loss values after 3 days and 8

days of chill storage and their significance are shown in Table 2.

After 3 days of chill storage, there was no difference in the amount of drip from stimulated and nonstimulated meat but the thawed meat, frozen at 6 or 24 hours, had about 8.2% drip compared with less than 1% for fresh meat.

After eight days of chill storage, more drip resulted from stimulated meat than from nonstimulated. In fresh meat and meat frozen at 24 hours, the increase in drip loss caused by electrical stimulation was slight (only 0.4%), but when frozen at 6 hours, electrical stimulation increased drip by about 3%. The drip loss in frozen and thawed averaged 11.4% and was considerably higher than in fresh meat which averaged 2.57%.

Calpains

Total calpains. The total calpain activities in fresh meat 1 hour post slaughter and in fresh and thawed meat after 1, 3 and 8 days of chill storage are shown in Table 3.

At 1 hour post slaughter and prior to freezing the activity was not significantly altered by electrical stimulation and averaged 50

Table 2. Drip loss in vacuum packs of fresh and thawed meat after 3 days and 8 days of chill storage. The means of 8 animals for each treatment expressed as a percentage of original weight. Mean square (MS) and significance (P) from ANOVA are given for stimulation (S), freezing (F), interaction (I) and residual (R).

2. tafla. Vökvatap (% af upprunalegri vigt) í fersku og þjódnu kjöti í lofttandum umbúðum eftir 3 og 8 daga kælingu. Meðalfervik (MS) og marktekt (P) eru sýnd fyrir raförvun (S), frýstingu (F), víxlhrif (I) og leif (R).

	F6 ^{a)}	F24 ^{b)}	Ch ^{c)}	S	MS and P		R
					F	I	
3 days							
NES	8.67	8.13	<1	0.9	358	1.3	5.5
ES	7.76	8.23	<1	NS	***	NS	
8 days							
NES	10.69	10.70	2.37	14.8	424	6.7	4.1
ES	13.29	11.03	2.76	*	***	NS	

a) Meat frozen at 6 hours—Kjöt frýst 6 klst. eftir slátnun.

b) Meat frozen at 24 hours—Kjöt frýst 24 klst. eftir slátnun.

c) Control fresh meat—Ófrýst kjöt.

Table 3. Total calpain activity in fresh meat 1 hour post slaughter and in fresh and thawed meat after 1, 3 and 8 days of chill storage. The means of 6 animals for each treatment expressed as units/g muscle and mean square (MS) and significance (P) from ANOVA for stimulation (S), freezing (F), interaction (I) and residual (R).

3. tafla. Heildarhvatavirkni kalpeins (einingar/g vöðva) í fersku kjöti 1 klst. eftir sláttun og í fersku og þíðdu kjöti eftir 1, 3 og 8 daga kælingu.

	F6	F24	Ch	S	MS and P		R
					F	I	
1 hour							
NES	54	52	47	39.4	24.4	86.6	80.8
ES	46	51	50	NS	NS	NS	
1 day							
NES	35	28	28	0	75.1	28.3	92.2
ES	31	30	29	NS	NS	NS	
3 days							
NES	22	22	33	14.3	378.0	17.2	56.9
ES	23	26	33	NS	**	NS	
8 days							
NES	26	23	29	6.8	92.8	0.9	80.4
ES	27	24	30	NS	NS	NS	

units/g. After 1 day of chill storage the average activity had fallen to 30 units/g and was unaffected by stimulation and freezing. After 3 days of chill storage in both stimulated and nonstimulated meat, meat frozen at 6 or 24 hours had 23 units/g, considerably lower than the fresh meat, which had 33 units/g. After 8 days of chill storage there was no significant difference between any of the treatments and the activity averaged 26.5 units/g.

Calpain I and II. At 1 hour post slaughter the activity of Calpain I in the L.D. was 44 units/g in nonstimulated and 32 units/g in stimulated meat and the activity of calpain II was 23 units/g in both stimulated and nonstimulated meat. The subsequent activities of calpain I and II are shown in Table 4. The activity of calpain I in nonstimulated meat fell to 12 units/g at 24 hours and then fell further to near zero at 3 and 8 days. In

Table 4. Activity of calpain I and II after 1, 3 and 8 days of chill storage.

4. tafla. Hvatavirkni kalpeins I og II eftir 1, 3 og 8 daga kælingu.

	Calpain I			Calpain II		
	F6	F24	Ch	F6	F24	Ch
1 day						
NES	7	4	12	26	29	22
ES	6	3	6	28	21	20
3 days						
NES	2	2	-2	15	21	34
ES	1	1	3	19	28	25
8 days						
NES	0	0	0	16	22	42
ES	-2	5	2	29	16	45

Table 5. Calpastatin levels of fresh and thawed meat after 24 hours of chill storage. Means of 2 animals for each treatment expressed as unit inhibitor/g muscle and the mean square (MS) and significance (P) from ANOVA for stimulation (S), freezing (F), interaction (I) and residual (R).

5. tafla. Kalpastatín í fersku og þíðu kjöti eftir 24 klst. í kælingu.

	Ch		F24		MS and P		R	
					F	I		
NES	100	49			220	3784	113	73
ES	82	46			NS	**	NS	

stimulated chilled meat the activity fell more rapidly to 6 units/g at 24 hours similar to that in frozen meat.

The exponential curves for the individual treatments did not differ significantly and a common rate constant (SE) for the fall in activity of calpain I was 0.085 (0.032) per hour.

There was little change in the activity of calpain II during storage up to 8 days. There was a tendency for the level to increase in chilled meat and decrease in frozen meat.

Calpastatin. The levels of calpastatin were significantly lower in the meat that had been frozen at 24 hours but were unaffected by stimulation (Table 5).

Cathepsins B and L. The total activities of cathepsins B and L, the activities in sarcoplasmic fluid and analysis of variance are shown in Table 6 and Table 7. The total activity was unaffected by freezing or stimulation and averaged 14.6 units/g. Freezing increased the activity in sarcoplasmic fluid by over 50%.

Cooking loss

The cooking losses of fresh and thawed meat

after 1 day, 3 days and 8 days of chill storage are shown in Table 8.

After 1 day of chill storage the cooking loss was 7% higher in thawed meat. The highest cooking loss, 29.2%, was in stimulated meat frozen at 24 hours and the lowest cooking loss, 20.0%, was in nonstimulated fresh meat. This 7% increase in frozen and thawed meat was reduced to 4% when cooked at 3 days and 3% when cooked at 8 days.

Texture

The texture after 1, 3 and 8 days of chill storage is shown in Table 9.

After 1 day of chill storage the highest shear force, 8.21 kg, was found in meat from stimulated carcasses frozen at 6 hours after slaughter while the lowest shear force, 4.15 kg, was found in meat from stimulated carcasses frozen at 24 hours. The electrical stimulation had a toughening effect in meat frozen at 6 hours while making meat frozen at 24 hours and fresh meat more tender.

Table 7. Analysis of variance. Mean square (MS) and significance (P) for cathepsin activity of the different treatments.

7. tafla. Fervikagreining á virkni katepsíns í kjöti eftir mismunandi meðferð.

Table 6. Activity of cathepsins B and L. Means of 2 replicates for each treatment.

6. tafla. Hvatavirkni katepsíns B og L.

	Total activity		% in sarcoplasmic fluid	
	F24	Ch	F24	Ch
NES	13.2	15.8	10.8	5.9
ES	14.6	14.6	10.6	6.8

	Total activity		% in sarcoplasmic fluid	
	M S	P	M S	P
Stimulation	0		0.3	
Freezing	4		37.7	*
Interaction	4		0.5	
Residual	11		2.1	

Table 8. Cooking loss in fresh and thawed meat after 1, 3 and 8 days of chill storage. The means of 8 animals for each treatment expressed as percentage uncooked weight together with mean square (MS) and significance (P) from ANOVA for stimulation (S), freezing (F), interaction (I) and residual (R). *8.tafla. Suðutap í fersku og þíðnu kjöti eftir 1, 3 og 8 daga kælingu.*

	F6	F24	Ch	S	MS and P		R
					F	I	
1 day							
NES	26.3	28.3	20.0	23.5	269.4	1.0	17.1
ES	27.7	29.2	21.9	NS	***	NS	
3 days							
NES	24.4	25.0	19.2	3.9	90.7	16.1	11.4
ES	25.1	23.5	21.6	NS	**	NS	
8 days							
NES	24.6	26.7	24.4	2.1	51.9	14.9	15.9
ES	25.8	26.7	21.9	NS	*	NS	

This interaction was significant at all testing times.

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After 3 days of chill storage the highest shear force, 5.33 kg was found in meat from stimulated carcasses frozen at 6 hours while the lowest shear force, 3.11 kg, was found in meat from stimulated carcasses frozen at 24 hours.

After 8 days of chill storage the highest shear force, 4.20, was found in fresh non-stimulated meat while the lowest shear force was still found in stimulated meat frozen at

24 hours.

The fall in texture was analysed by the MLP program using an exponential decay equation and showed that the curves for the individual treatments did not differ signifi-

cantly and a common rate constant (SE) for the individual curves was found to be 0.015 (0.004) per hour. The displacement of the curves was significant, the largest difference initially between stimulated meat frozen at 6 hours, 8.2 kg, and stimulated meat

Table 9. Texture of fresh and thawed meat after 1, 3 and 8 days of chill storage. The means of 8 animals for each treatment expressed as kg shear force together with the the mean square (MS) and significance (P) from ANOVA for stimulation (S), freezing (F), interaction (I) and residual (R). *9. tafla. Seigja í fersku og þíðnu kjöti eftir 1, 3 og 8 daga kælingu.*

	F6	F24	Ch	S	MS and P		R
					F	I	
1 day							
NES	6.59	5.17	6.71	0.06	30.4	8.6	2.1
ES	8.21	4.15	5.90			*	
3 days							
NES	4.73	4.24	5.15	2.1	8.3	3.3	1.0
ES	5.33	3.11	4.41			*	
8 days							
NES	2.78	2.90	4.20	0	7.6	1.8	0.5
ES	3.56	2.45	3.88			*	

frozen at 24 hours, 4.2 kg.

DISCUSSION

In countries with an abundance of lamb at certain times of the year, large quantities of meat have to be frozen for later consumption. If lamb were to be thawed on an industrial scale and presented in the unfrozen state to the consumer with similar quality, in terms of tenderness, as required for fresh lamb by the major supermarkets, it is important to know about the texture values and drip losses involved and how electrical stimulation affects them. In order to understand better the mechanism of tenderisation of meat that has been frozen and thawed it is necessary to measure the enzyme levels of thawed meat.

The objective of this work was to study the effects of low-voltage electrical stimulation and freezing at 6 hours or 24 hours post slaughter on tenderisation, enzyme levels, drip losses and cooking losses in lamb. In order to do this nonstimulated and stimulated lamb was held at 10°C for 6 hours or 24 hours post slaughter and then frozen. After thawing 7 days later this lamb was compared, during chill storage at 2°C for 7 days, with stimulated and nonstimulated fresh lamb, that had been held at 10°C for 24 hours before commencement of chill storage at 2°C. This approach enables the expression

of the importance of freezing, frozen storage and thawing as the biochemical and quality changes can be studied and compared to those in the fresh non-frozen product. For clarity of comparisons thawed meat after 1 day of chill storage refers to immediately upon thawing, i.e. chill storage for thawed meat refers to storage in the non-frozen state.

Electrical stimulation

Low-voltage electrical stimulation applied during bleedout effectively reduced the pH values of the L.D. at 1 hour and 6 hours after slaughter. The pH of the stimulated carcasses had already reached 6.2 within 1 hour of slaughter and by 6 hours, was only 0.1 unit

above the ultimate pH of 5.6. The pH of the nonstimulated carcasses was a little over 6.2 at 6 hours.

The rates of pH fall in the L.D. in this study are similar to those obtained by Chrystall and Devine (1985) using low-voltage stimulation of lamb carcasses held at 10°C, although their stimulation tended to be less effective with the pH at 1 hour and 6 hours about 0.2 units higher than obtained in this work. The same authors obtained a more rapid pH fall by employing high voltage stimulation when the pH reached 6 within 2 hours compared to 3.5 hours by low-voltage stimulation.

Although the pH was measured only in the L.D. the pH of this muscle is a good indication of the pH of the major muscles in the carcass (Chrystall and Devine, 1985).

Electrical stimulation is a valuable process to reduce holding times for lamb in the meat industry. More work has been done on the effects of high-voltage than low-voltage stimulation. Low-voltage stimulation, employing less than 100 V, has the advantage that installation costs are low and the equipment demands little space but the disadvantages are more variable results, the process can not be automated and has to be applied within minutes of slaughter. High-voltage stimulation, employing often more than 500 V, has the advantage that the effects are very reproducible, the process can be automated and applied up to 50 min after slaughter, but the installation costs are high, mainly

because of necessary safety precautions, and the equipment demands considerable space which may not be available in slaughterhouses not designed with the process in mind. The application of low-voltage electrical stimulation should therefore be a feasible alternative in older slaughterhouses where throughput is low.

Cooling

Three cooling treatments were used: non-frozen control and freezing at 6 hours and

24 hours post slaughter.

Chilling at 10°C commenced at 1 hour after slaughter at which time the temperature in the deep leg was 38.5°C. After 6 hours the deep leg had reached 17°C and at 24 hours after slaughter the temperature was 10.5°C. In non-frozen meat the loins were then chilled at 2°C. In meat frozen at 6 hours after slaughter the temperature in the meat reached 10°C about 1 hour after entry into the freezer (7 hours after slaughter) when the pH of stimulated carcasses was about 5.7 and nonstimulated 6.2. In nonstimulated carcasses, chilled slowly, the temperature at 6 hours was about 17°C when the pH was 6.2 and would induce only the minimum of muscle shortening. With electrical stimulation the temperature was between 17°C and 38°C when entering rigor. Bearing in mind that stimulated carcasses enter rigor at 30°C

(Chrystall and Devine, 1985) and ageing starts at rigor onset, then much of the ageing process will occur more rapidly at the relatively higher temperature. In stimulated sides, frozen at 6 hours, the temperature was 10°C when the pH was 5.7 and cold shortening toughness is unlikely to occur except in surface muscles.

Freezing

Slow chilling was followed by relatively fast freezing at 6 hours and 24 hours. For meat frozen at 6 hours the deep leg temperature reached -4°C 13 hours from slaughter. More rapid initial cooling in New Zealand has

shown that when the deep leg temperature of stimulated carcasses falls below -4°C in less than 14 hours from slaughter the tenderness of legs and loins is less than desirable (Chrystall and Hagyard, 1975; Chrystall, 1980). The frozen loins were kept frozen at -20°C for 7 days to simulate commercial frozen storage.

Thawing

Thawing was done at a chill temperature of 7°C to simulate the relatively slow thawing

likely to occur in commerce. Creed *et al.* (1979) found optimal thawing conditions, in terms of microbiological quality and drip losses, for unwrapped lamb carcasses 7.5°C and air velocity 0.75 m/s for 24 hours.

The meat was thawed as vacuum packed

loins at 7°C in 24 hours. Thawing time from -2°C to -1°C took approximately 11 hours and the mean temperature of the L.D. after the 24 hour thawing period was 0.5°C. In whole carcasses a longer thawing time would be expected as the legs and the forelimb are thicker than the loin and would therefore take a longer time to thaw.

Drip

The drip losses of frozen and thawed meat were significantly higher than those of fresh meat at 3 and 8 days after slaughter. This was to be expected. The increased drip loss in thawed meat is because of ice crystal formation and mechanical breakdown of tissue structures in the muscle during freezing (Voyle, 1974). The drip losses of fresh meat increased from less than 1% at 3 days post slaughter to about 2.6% 8 days post slaughter and the drip losses of thawed meat from about 8.2% to 11.4%. These losses are considerable and might make commercial thawing of meat for retail sale uneconomical but the drip losses were measured in relatively small cuts of the L.D. (70-170 g) and as the drip losses are proportional to the cut surfaces to the weight of muscle those found in this experiment are relatively large. The drip losses are further increased by vacuum packing which exerts pressure on the meat, squeezing out more drip. The drip losses from whole carcasses are negligible but drip accumulates in endomysial and perimysial spaces of the muscle (Offer *et al.*, 1989) and exudes as drip when the muscles are cut.

Electrical stimulation only had a significant effect on drip losses in early frozen meat after 8 days chill storage where there was a 2% increase compared with nonstimulated meat. This increase was probably due

to the rapid pH fall while the temperature was high prior to cooling. In meat frozen at 24 hours and fresh meat the increase caused by electrical stimulation after 8 days chill storage was slight (0.3–0.4%). Reports differ on the effect of electrical stimulation on drip losses. Some workers have found an increase in drip loss with stimulation, others a decrease. According to Bendall (1980) the increase in drip losses from beef and lamb carcasses caused by electrical stimulation are usually not significant and within acceptable limits of 5% in fresh meat. In this study the highest drip loss found in fresh meat was 2.76% and well within acceptable limits. There are mainly two reasons for increased drip loss, shortening of sarcomere

length, resulting in a tighter filament lattice, or fast glycolysis and slow chilling, resulting in a denaturation and shortening of the myosin heads and a tighter filament lattice (Offer *et al.*, 1989). Chilling was faster in the early frozen group so increased drip loss in this group was most likely due to some shortening of sarcomere length.

Enzymes

Several enzymes are thought to be responsible for changes in texture. The principle ones are calpains and cathepsins (Etherington, 1984; Penny, 1980). The activities of these enzymes were measured *in vitro* under optimal conditions and these are therefore a measure of the potential activities of these enzymes in meat.

Calpains. Total calpain activity in nonstimulated fresh meat at 1 hour post slaughter was on average 51 units/g meat. This activity is about 40% lower than the activity in lamb found by Etherington *et al.* (1987). The calpain I activity of fresh nonstimulated meat at 1 hour post slaughter in this work was found to be 44 units/g meat and that is about 20% lower than the activity found by Etherington *et al.* (1987).

Levels of total calpains were not significantly affected by freezing except at 3 days

after slaughter when they were lower than in fresh meat. After 1 day chill storage (upon thawing) the total calpain activity of early frozen meat was higher than in fresh meat and meat frozen at 24 hours. After 3 days chill storage the total calpain activity of thawed meat had fallen to a level that was significantly lower than in fresh meat. After 8 days of chill storage the levels of total calpains in fresh and thawed meat were not significantly different. Koolmaraie (1990a) found calpain I and calpain II stable in meat pieces frozen by liquid nitrogen and stored at -70°C . There seemed to be only small losses of total calpains during freezing, frozen storage and thawing. However, the activity of calpain I was 2–3 times higher in fresh meat after 1 day of chilled storage than in meat frozen at 24 hours upon thawing indicating that activity of calpain I was selectively lost during freezing, frozen storage and thawing while the activity of calpain II was unaffected.

The levels of total calpains appeared to be little affected by electrical stimulation but the levels of calpain I were found to be about 40% lower at 1 hour after slaughter in stimulated meat and at 3 days about 50% lower in fresh stimulated meat. Etherington *et al.* (1990) found calpain I significantly lower in stimulated than nonstimulated chicken meat and Dransfield *et al.* (1990) found a faster loss of activity of calpain I in stimulated than nonstimulated beef. This is probably because electrical stimulation will release Ca^{2+} -ions earlier, while the temperature in meat is high, causing earlier autolysis of calpain I but calpains are subject to autolysis in the presence of calcium (Guroff, 1964).

The levels of total calpains fell during chill storage. This fall was rapid in the first 24 hours and thereafter the activity was relatively stable in fresh meat whereas in meat that had been frozen there was a further decrease in total calpain activity after thawing. This effect could possibly be because of

an increase in intracellular calcium caused by freezing since calpains are subject to autolysis in the presence of calcium (Guroff, 1964) or lower levels of the inhibitor, calpastatin. Calpain I was preferentially lost during chill storage and this is in agreement with the findings of other authors (Ducastaing *et al.*, 1985; Etherington *et al.*, 1990; Koochmaraie *et al.*, 1987). The decay constant for the fall of calpain I in fresh and frozen meat was found to be 0.085 per hour. The levels of calpain II remained fairly constant throughout the period of chill storage although there was a tendency for higher values to be found at later times. As the activity is calculated from the relative activities of calpain I and calpain II this suggests that the activity of calpain I is somewhat higher than it was calculated or calpain I activity was lost during the separation by FPLC. This relatively small loss of calpain I activity could have considerable influence on the calculation of the exponential decay rate of calpain I and the rate constant.

Calpastatin. The levels of the inhibitor calpastatin were reduced by about 50% in frozen and thawed meat compared to fresh meat. Koochmaraie (1990a) found the inhibitor unstable in small meat pieces frozen in liquid nitrogen. He found a 34% loss of activity during 2 weeks storage at -70°C and 55% loss of activity during 6 weeks storage. The loss of activity found during one week at -20°C in this study is considerably greater. In commercial freezing, then, the losses in calpastatin are likely to be much greater than hitherto suspected.

The levels of calpastatins were not significantly affected by electrical stimulation but the levels of the inhibitor were lower in stimulated than nonstimulated fresh meat. Ducastaing *et al.* (1985) found that electrical stimulation lowered inhibitor activity at 24 hours by 40% in beef muscle compared with the control which is a considerably greater decrease in activity than that found in this study which was about 20% less in stimu-

lated fresh meat.

Cathepsin B and L. The activity of cathepsin B and L "free" in sarcoplasmic fluid was increased by 50% in thawed meat compared to fresh meat, whereas there was virtually no change in the total activity of these enzymes in muscle by freezing. Cathepsins have been found to be stable in meat during storage and freezing and thawing will release cathepsins from the lysosomes (Davies, 1975) presumably by rupture of the lysosomal membranes by ice crystal formation.

The total and "free" levels of cathepsins B and L were not significantly affected by electrical stimulation. This disagrees with

the findings of Etherington *et al.* (1990) who showed significantly higher cathepsin B and L levels in stimulated than nonstimulated chicken meat. However, it is difficult to explain how total cathepsin B and L activity can increase by electrical stimulation although higher levels of cathepsin B and L "free" in sarcoplasmic fluid can increase, presumably because of rupture of lysosomal membranes by stimulation (Dutson *et al.*, 1980).

Cooking loss

The cooking loss was significantly affected by freezing at all observation times, being up to 10% higher after 1 day of chill storage

in thawed stimulated meat than in fresh nonstimulated meat. The cooking loss was not affected by electrical stimulation. The cooking losses were found to increase during chill storage in fresh meat. It was highest after 8 days of chill storage in nonstimulated meat over 24%. On the other, hand the cooking losses of thawed meat were highest upon thawing and lower, on average 2.7%, after 3 days and 8 days of chill storage.

Texture

Freezing had a tenderising effect on meat frozen at 24 hours but a toughening effect on meat frozen at 6 hours. This interaction was significant at all observation times. The

rate of tenderisation did not differ significantly between the treatments and supports the results that none of the groups had cold-shortened meat. Meat frozen at 24 hours was considerably more tender upon thawing than fresh meat. This is similar to the results of Winger and Fennema (1976) and Locker and Daines (1973) but they found a decrease in toughness after one freeze-thaw cycle of beef. The texture of meat frozen at 24 hours was similar upon thawing to the texture of fresh meat after 3 days of chill storage and, since the rate of tenderisation was not different, most of this difference seems to be a mechanical damage resulting from ice crystal formation (Voyle, 1974). To prove this, aged meat (from the fresh group of the last two sets of animals) was frozen after 8 days chill storage, kept frozen for 1 week and thawed at 7°C. This freeze-thaw cycle decreased shear force of nonstimulated meat by 15% and decreased shear force of stimulated meat by 26%. Locker and Daines (1973) found a decrease in shear force of 8% after one freeze-thaw cycle in beef but that was fast freezing and thawing under laboratory conditions.

Low-voltage electrical stimulation had a tenderising effect on fresh meat and meat frozen at 24 hours but a toughening effect on meat frozen at 6 hours. This interaction was significant but was mostly due to freezing. There have been some reports of a toughening effect of electrical stimulation. Takahashi *et al.* (1984) showed that low-frequency stimulation reduced pH but had a toughening effect in beef. Marsh *et al.* (1987) showed that an intermediate rate of post-mortem glycolysis gave the most tender meat and, when the chilling rate of beef carcasses was very low, electrical stimulation resulted in tougher meat. This effect can, however, not be the cause of the toughness of early frozen meat encountered in this experiment because the toughening effect would have been the same or even greater in fresh meat or meat frozen at 24 hours which had a slower chilling rate than meat frozen at 6

hours. This effect, therefore remains unexplained.

The texture values decreased by an average of about 45% from day 1 to day 8 of chill storage. The exponential rate constant of fall in texture was found to be 0.015 per hour or 0.36 (0.1) per day. The rate of the curves for the different treatments was the same, only the displacement of the curves was different. The rate constant found in this study is somewhat higher than the rate constant of 0.21 (0.05) per day for lamb found by Dransfield *et al.* (1980) but was much lower than the rate of decay of calpain I found in this work. Commercial freezing rates and slow thawing did not increase the rate of tenderisation in this work but Dransfield (1986) and Locker and Daines (1973) found an increased rate of tenderisation by rapid freezing and thawing.

According to Rhodes (1972) a shear force of 5 kg would be judged by a trained taste panel as slightly to moderately tender and that value should be the upper limit for a mean of 8 carcasses because a higher mean score would include carcasses that were characterised as tough. Stimulated meat frozen at 24 hours is already under this limit upon

thawing and nonstimulated meat very near it. After 3 days chill storage only nonstimulated fresh meat and stimulated meat frozen at 6 hours are slightly over this limit.

Although the texture was measured only in the L.D. it is a good indication of the quality of the major muscles in the carcass. In experiments with different holding times before freezing, the incidence of acceptable tenderness for different treatments was always higher in the legs than the loins (Chrystall and Devine, 1985).

General discussion

There has been considerable interest the last few years in the relative importance of the different muscle proteinases in the tenderisation process of meat. Koochmarai (1990b) is of the opinion that calpains play a key

role in tenderisation of meat but other workers (Etherington, 1984; Dutson, 1983) have argued that it could be the lysosomal cathepsins that are at least partly responsible for the tenderisation process.

In this work the rate of fall in texture was lower than the rate of fall of calpain I. This indicates that calpain I was not solely responsible for tenderisation. The same can be said of the fall in levels of the inhibitor, calpastatin, caused by freezing. Despite lower levels of calpastatin in thawed meat the rate of tenderisation was not increased. Despite a 50% increase in cathepsin B and L levels "free" in sarcoplasmic fluid after freezing and thawing, the rate of tenderisation of thawed meat was no faster than the rate of fresh meat.

Meat held at 10°C and frozen in 24 hours is already tender upon thawing without any further conditioning and, when stimulated, very tender. In countries that have to freeze large quantities of meat for preservation it should be possible to thaw meat in 24 hours, butcher it in a conventional manner and present it in the non-frozen state immediately upon thawing but with considerable drip losses. Meat frozen at 24 hours could be distributed and sold immediately but meat frozen at 6 hours would have to be conditioned for at least 2 days after thawing to be acceptably tender and this would involve more drip losses, but by freezing early there are lower evaporation

losses and lower requirements for chiller space. However, evaporation losses of lamb carcasses can only be expected to be 2-3% during the first 24 hours after slaughter in chill storage of which about half is lost during the first 2-3 hours after slaughter (Cutting, 1973). The scale of the potential savings in evaporation losses, by freezing early, would certainly be outweighed by an increase in drip losses if acceptable tenderness of early frozen meat was to be achieved by ageing after thawing.

CONCLUSIONS

The rate of tenderisation in thawed meat was found to be the same as in fresh meat.

Meat frozen at 24 hours post slaughter was already acceptably tender after thawing at 7°C for 24 hours and if stimulated by low-voltage electrical stimulation very tender. Fresh meat and meat frozen at 6 hours reached the level of acceptable tenderness after a further 2 days chill storage.

The total calpain activity appeared to be little affected by freezing but the rate of fall in activity was higher after thawing.

The activity of calpain I was found to be decreased by freezing, frozen storage for 1

week and thawing while the activity of calpain II was unaffected.

The activity of calpain I was found to be decreased by electrical stimulation while the activity of calpain II was unaffected.

The activity of the inhibitor, calpastatin, was found to be reduced by 50% by freezing, frozen storage for 1 week and thawing.

The activities of cathepsin B and L in sarcoplasmic fluid were found to be increased by 50% by freezing and thawing whereas the activity in muscle extracts did not change.

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