EFFECT OF RAPID POSTMORTEM TEMPERATURE DROP ON TENDERNESS AND AGING OF MEAT FROM LAMBS OF DIFFERENT AGE.

I.JAIME, J.A.BELTRAN, P.CEÑA, P.LOPEZ-LORENZO and P.RONCALES

Dep. Producción Animal y Ciencia de los Alimentos. Facultad de Veterinaria. Universidad de Zaragoza. SPAIN.

INTRODUCTION

It has been shown repeatedly that conditions at which carcasses are held postmortem exert a doubtless influence on meat quality. This is particularly evident when the effect of temperature on meat tenderness is considered.

It is generally accepted that low temperatures cause cold shortening to appear (Locker and Hagyard, 1963; Honikel et al., 1983) as well as it was thought, too, that this was directly related to meat thoughening (Marsh and Leet, 1966). Though it seems likely that shortening itself is not sufficient to cause toughening, since it has been shown that muscles with similar sarcomere length can reach very different tenderness scores (Dutson et al., 1977; Culler et al., 1978). On the other hand, some authors found tender meats when carcasses were held at high temperatures (37^{0}C) either along rigor development (Petaja et al., 1985) or over a short period early postmortem (Lochner et al., 1980; Marsh et al., 1980-81). It has been however more recently reported that high temperature together with a low pH give rise to very tough meat (Marsh et al., 1987).

It is then a fact that controversy still exists on the effect of muscle postmortem final temperature reached, as well as the rate of its decrease, upon meat tenderness. Besides this, research on this subject using lamb carcasses is scarce if that related to electrical stimulation is not considered (Marsh et al., 1968; McCrae et al., 1971; Bouton et al., 1971; Bowling et al., 1978).

We have studied the effect of holding lambs *Longissimus dorsi* muscles at different temperatures, so as to reach within 3-4 hours postmortem internal temperatures of either 0^0, 4^0, 10^0, 15^0, 20^0 or 36^{0}C, upon meat tenderness measured over 7 days of aging. In order to assess the foreseeable influence of lambs age on meat characteristics, two groups of animals were used throughout the study: 9-12 Kg and 16-20 Kg carcass weight.

MATERIALS AND METHODS

Pairs of *Longissimus dorsi* muscles obtained from lambs of 9-12 Kg or 16-20 Kg carcass weight were excised from carcass within 30 min postmortem. They were immediately trimmed of visible fat, wrapped in polyethylene bags and brought to internal temperatures of either 0^0, 4^0, 10^0, 15^0, 20^0 or 36^{0}C in a water bath. One muscle of each pair was used for measuring pH and degree of shortening until rigor onset, while internal temperature was continuously monitored using a termocouple inserted into the middle of the corresponding muscle from the other carcass side. After rigor was completed, the latter was stored in a cooler and aged for 7

days at a constant temperature for all prerigor treatments of 4°C. At least three pairs of muscles were used for each conditioning temperature treatment.

Measurement of shortening. Several bundles of muscle fibers of about 0.3 cm diameter and 5 cm length were excised from whole muscles within one hour postmortem, before submerging them into the conditioning bath, and their exact length was measured. The bundles were then kept in the bath until rigor onset, when length was measured again. Shortening is expressed as the percentage of the difference between initial and final lengths related to initial value.

pH of tissue. About 3 g of muscle were homogenised in 20 ml distilled water for 15 s. The measurement was carried out immediately using a Crison pH-meter and a combined glass electrode.

Tenderness evaluation. A ten-member semi-trained taste panel evaluated samples for overall tenderness using a 9-point scale; 9 denoted extremely tender and 1 denoted extremely tough. After muscle was trimmed of visible fat and connective tissue, 0.7 cm thick steaks were cut transverse to muscle axis and fried with very little oil on a frying pan to an internal temperature of 70°C. Steaks were divided in four sections and two of them, selected at random, presented to each panel member for evaluation.

SDS-gel electrophoresis. We employed a modification of Laemmli procedure according to Greaser et al.

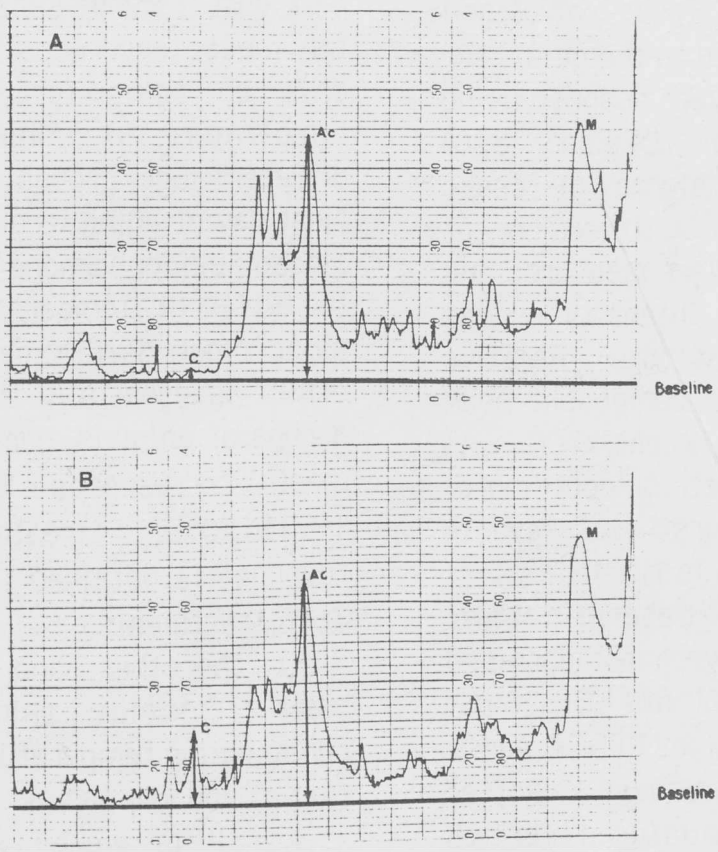

Figure 1.- Example of densitometric recording of myofibrillar proteins from lamb *Longissimus dorsi* muscle separated by SDS-PAGE. The peaks are: M: myosin, Ac: actin and C: 30000 D component. A: first day of aging, B: seventh day of aging.

(1983). This system uses a 1.5 mm thick slab gel consisting of a 15% polyacrylamide resolving gel and 3% stacking gel. The acrylamide:bisacrylamide crosslinker ratio is 200:1 in the resolving gel and 20:1 in the stacking gel. Electrophoresis was performed at a constant voltage of 120 V at the stacking gel and then at 250 V for about 5 h. Gels were stained using a solution of Coomassie blue R 250 and destained until background was clear. Myofibrils were essentially prepared according to the procedure dsecribed by Olson et al. (1976). Myofibril suspensions were dissolved in 0.05 M Tris-HCl bufffer (pH 6.8) and boiled for 5 min in presence of 6% SDS. After heating, 40% sucrose was added in a ratio of 1:3 (v/v) and the final protein concentration was adjusted to 10 mg/ml. 10 µl of these myofibril solutions were applied to the gels.

After drying gels were scanned using a Kontron (Model Uvikon 820) densitometer. The heights of peaks corresponding to 30000 D component band and actin band were measured and the proteolysis intensity was expressed as following: Relative intensity = (30000 D component peak height/actin peak height)x100. An example of densitometric recording of gels obtained the first and seventh days of aging is given in fig. 1

Free calcium determination. Three g of muscle were homogenised with 10 ml buffer (100 mM KCl, 20 mM K posphate pH 7.0, 1 mM EDTA and 1 mM sodium azide) using a Potter homogeniser and centrifuged for 15 min at 1000xg. The supernatant was decanted, the pellet was resuspended in 10 ml of the same buffer and centrifuged again for 15 min at 1000xg. Both supernatants were mixed, 8 ml of the mixture were then removed, treated with 1 ml of 6.4% TCA. and after 1 h centifuged for 1 h at 1000g. The supernatant was decanted and1% lanthinium oxide was added to it. Calcium was determined using a IL-S11 Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

Muscle final temperature for each treatment is reached within 3-4 hours postmortem in all cases and it is held constant until the onset of rigor mortis (results not shown). Thus most time required by muscles to undergo the biochemical changes which lead to rigor occur at the desired temperature of conditioning.

Figure 2 shows sensory panel scores for meat tenderness at 1st and 7th days of aging for each treatment temperature in animals of the two different age groups studied. On the first day of aging large differences are already evident among muscles subject to different temperature treatments along rigor development. Temperature effect is esentially the same in both groups of animals; namely most tender meat corresponds to $0^{o}C$ and $10-15-20^{o}C$ treatments, while toughest is obtained following 4^{o} and $36^{o}C$ treatments.

On the other hand, as foreseeable, meat from older animals is for all conditioning temperatures tougher than that of younger lambs.

As it is well known muscle shortening plays an important role in meat toughening, beeing early postmortem conditioning temperature

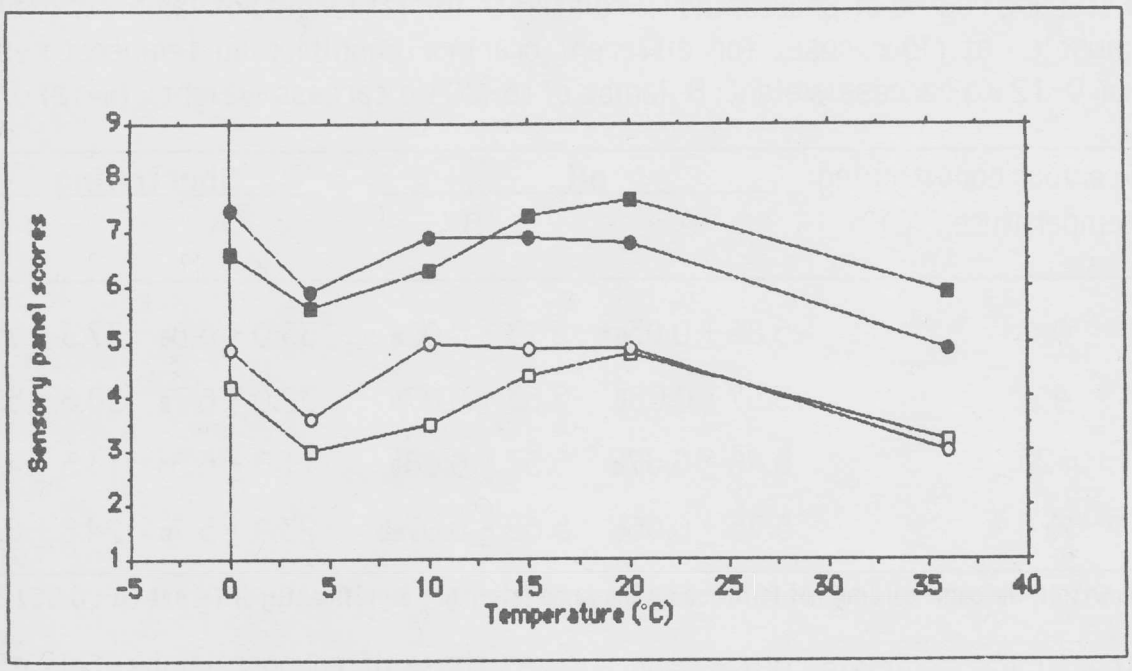

Figure 2.- Overall tenderness panel scores for lamb meat aging for 7 days after different prerigor conditioning temperature treatments. First day of aging: lambs of 9-12 Kg (○) and 16-20 Kg (□) carcass weight; seventh day of aging: lambs of 9-12 Kg (●) and 16-20 Kg (■) carcass weight.

determinant of the degree of shortening undergone by muscles. It can be observed in Table 1 that both low and high temperatures cause a considerable shortening to occur. Shortening induced by lower temperatures (cold shortening) has been extensively studied in cattle (Locker and Hagyard, 1963; Honikel et al., 1983) as well as in lamb and mutton (McCrae et al., 1971; Bowling et al., 1978); our results essentially agree with those previously reported by those authors.

When one compares the results depicted in Fig. 2 and Table 1, it appears evident that most tender meat corresponds to lesser muscle shortening while toughest meat corresponds to strongest shorthening, with the sole exception of $0^{o}C$ treatment in both different age animal groups, in which tender meat is obtained from strongly shortened muscles. This result appears to be inconsistent with previous reports (Marsh et al., 1968; McCrae et al., 1971) in which cold shortening was shown to be directly related to meat toughness. In no prior case was reported however such a rapid postmortem muscle temperature drop as it is here described, nor its effect on lamb meat tenderness.

Final pH reached after each conditioning temperature is also shown in Table 1. It is highly noticeable that pH at rigor onset for $0^{o}C$ treatment is significantly higher than those reached at temperatures above until those near physiological. Thus high pH seems to be related to meat tenderness, even in shortened muscles, as was previously shown by Bouton et al. (1971) for lamb meat, as well as

Table 1.-pH and degree of shortening (% related to initial length) of lamb *Longissimus dorsi* muscle at rigor onset for different prerigor conditioning temperatures. (A: lambs of 9-12 Kg carcass weight; B: lambs of 16-20 Kg carcass weight). **(n=12)**

Prerigor conditioning temperature (^{0}C)	pH		Shortening	
	A	B	A	B
0	5.85 ± 0.05a*	5.73 ± 0.02a	33.0 ± 6.8a	37.3 ± 7.3a
4	5.57 ± 0.05b	5.63 ± 0.07b	32.0 ± 6.7a	30.8 ± 5.0a
10-20	5.48 ± 0.07b	5.57 ± 0.08b	11.3 ± 6.5b	13.5 ± 4.8b
36	5.75 ± 0.06c	5.68 ± 0.07ab	23.9 ± 5.7c	24.8 ± 8.1c

*** Means within the same column not followed by the same letter are significantly different ($p < 0.05$).**

for other meat animals (Khan and Nakamura, 1970; Bouton et al., 1973; Marsh et al., 1980-81).

As may also be observed in Fig. 2 tenderness increases along aging at a similar rate for all studied temperature treatments, so that first day differences among treatments, appear to remain unchanged after six days of storage. Though, tenderisation is no doubt further achieved for all prerigor conditioning temperatures in the older animals group, since tougher meats at first day of aging become approximately so tender at seventh day as those of younger lambs. This effect could be due to a higher proteolytic activity existing in more mature muscles of older and heavier animals.

In order to relate the proteolytic activity upon myofibrillar proteins to the tenderising effect of meat aging, SDS-polyacrylamide gel electrophoresis of muscle proteins were carried out at 1st and 7th day of aging (electrophoretic patterns not shown). Since the appearance and height increase of a 30000 Dalton component peak along aging is the most noticeable and steady change denoting proteolytic

Table 2.- Proteolytic rate (expressed as the percent ratio 30000 D component peak height to actin peak height of corresponding SDS-PAGE densitograms) of lamb *Longissimus dorsi* at 1st and 7th days of aging for different prerigor conditioning temperatures (A: lambs of 9-12 Kg carcass weight; B: lambs of 16-20 Kg carcass weight).

Prerigor conditioning temperature (^{0}C)	A		B	
	1st	7th	1st	7th
0	23.9	49.3	19.7	72.3
4	7.9	25	0	33
10-20	11	22	9.4	35.1

activity, this has been estimated by calculating the percent ratio of 30000 Dalton component peak height to actin peak height as described before.

Table 2 shows proteolytic rates so estimated for three groups of temperature treatments and different animal age. It can be first observed that values of proteolytic rate are higher in all cases for lambs of 16-20 Kg carcass weight, which agree with our results showing a more intense tenderising effect of aging on older animals meat. Besides this, differences can also be appreciated among the various conditioning temperatures studied, which are similar for both animal groups. Proteolysis appears to be earlier and more intense in meats conditioned early postmortem at 0^0C, which is likely to be responsible for a deepest tenderising effect capable of even overcoming initial toughness due to cold shortening. Meats conditioned at either 4^0C or 10^0-15^0-20^0C show similar proteolytic rate, which results in different meat tenderness since those stored postmortem at 4^0C suffer a stronger shortening than those held at higher temperatures.

Higher proteolytic activity of CAF in muscles held at 0^0C postmortem could be only in part referred to higher pH reached at rigor onset (Dayton et al., 1976; Goll et al., 1983; Koohmaraie et al., 1986), when compared to other temperature treatments. In a previous report on the subject (Beltrán et al, 1986) we ventured the prospective influence of increased sarcoplasmic Ca^{++} levels by effect of low temperatures on CAF activity. Table 3 shows free Ca^{++} concentration at 5 hours postmortem and at rigor onset for three representative temperatures of prerigor conditioning.

According to the results obtained, and in agreement with Kanda et al. (1977) and Cornforth et al. (1980), low temperatures bring about a considerable release of Ca^{++} from sarcoplasmic reticulum to the myofibrils very early postmortem, especially important at a temperature as low as 0^0C. This early supply of free Ca^{++}, together with a high muscle pH,

Table 3.- Effect of prerigor conditioning temperature on free Ca^{++} concentration (M)[a] in *Longissimus dorsi* muscle of 9-12 Kg carcass weight lambs at 5 hours postmortem and rigor onset.

Prerigor conditioning temperature (^{0}C)	5 h postmortem	Rigor
0	1.5×10^{-5}	3.7×10^{-5}
4	7.2×10^{-6}	3.0×10^{-5}
15	1.0 x 10-7	2.5 x 10-5

[a]Only proximate values are given since data obtained were corrected by substracting to all of them a fixed amount of Ca^{++} corresponding to that released from sarcoplasmic reticulum during muscle homogenisation, as evidentiated by a S.R. marker enzyme activity (not shown).

can very reasonably explain an activation of CAF and intense tenderisation capable of overcoming toughness caused by cold shortening.

CONCLUSIONS

Early postmortem conditioning of *Longissimus dorsi* from lambs of different age at low (< 10^0C) or high temperatures (> 20^0C) causes muscle to shorten, which results in meat toughening except for very low (~0^0C) temperatures. This fact is referred to a very early and higher proteolytic activity in muscles held at very low internal temperatures, stimulated by both high pH even at rigor onset and the release of large amounts of Ca^{++} from sarcoplasmic reticulum very early postmortem.

ACKNOWLEDGEMENTS

This research was supported by grants of Diputación General de Aragón (ref. no. A1.5/84) and Universidad de Zaragoza (ref. no. IN1/88).

REFERENCES

Beltrán, J.A., Jaime, I., Lopez-Lorenzo, P. & Roncalés, P. (1986):
Effect of early conditioning temperature on tenderness and aging of lamb meat. Proc. 32nd Eur. Meet. Meat Res. Work. 1:157-159.

Bouton, P.E., Carrol, F.D., Harris, P.V. & Shorthose, W.R. (1973):
Influence of pH and fiber contraction state upon factors affecting the tenderness of bovine muscle. J. Food Sci. 38:404-407.

Bouton, P.E., Harris, P.V. & Shorthose, W.R. (1971):
Effect of ultimate pH upon the water-holding capacity and tenderness of mutton. J. Food Sci. 36:435-439.

Bowling, R.A., Smith, G.C., Dutson, T.R. & Carpenter, Z.L. (1978):
Effects of prerigor conditioning treatments on lamb muscle shortening, pH and ATP. J. Food Sci. 43:502-507.

Cornforth, D.P., Pearson, A.M. & Merkel, R.A. (1980):
Relationship of mitochondria and sarcoplasmic reticulum to cold shortening. Meat Sci. 4:103-121.

Culler, R.D., Parrish, F.C., Smith, G.C. & Cross, H.R. (1978):
Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43:1177-1180.

Dayton, W.R., Reville, N.J., Goll, D.E. & Stromer, M.H. (1976):
A calcium-activated protease possibly involved in myofibrillar protein turnover. Partial characterization of the purified enzyme. Biochemistry 15:2159-2167.

Dutson, T.R., Yates, L.d., Smith, G.C., Carpenter, Z.L. & Hostetler, R.L. (1977):
Rigor onset before chilling. Ann. Rec. Meat Conf. Proc. 30:79-86.

Goll, D.E., Otsuka, Y., Nagainis, P.A., Shannon, J.D., Sathe, S.K. & Muguruma, M. (1983):
Role of muscle proteinases in maintenance of muscle integrity and mass. J. Food Biochem. 7:147-177.

Greaser, M.L., Yates, L.D., Krzywicki, K. & Roelke, D.L. (1983):
Electrophoretic methods for the separation and identification of muscle proteins. Ann. Rec. Meat Conf. Proc. 36:87-91.

Honikel, K.O., Roncales, P. & Hamm, R. (1983):
The influence of temperature on shortening and rigor onset in beef muscle. Meat Sci. 8:221-241.

Kanda, T., Pearson, A.M. & Merkel, R.A. (1977):
Influence of pH and temperature upon calcium accumulation and release by bovine sarcoplasmic reticulum. Food Chem. 2:253-266.

Khan, A.W. & Nakamura, R. (1970):
Effects of pre and postmortem glycolysis on poultry tenderness. J. Food Sci. 35:266-267.

Koohmaraie, M., Schollmeyer, J.E. & Dutson, T.R. (1986):
Effect of low-calcium-requiring Calcium Activated Factor on myofibrils under varying pH and temperature conditions. J. Food Sci. 51:28-32.

Lochner, J.V., Kauffman, R. & Marsh, B.B. (1980):
Early postmortem cooling rate and beef tenderness. Meat Sci. 4:227-241.

Locker, R.A. & Hagyard, C.J. (1963):
A cold shortening effect in beef muscles. J. Sci. Food Agric. 14:787-793

Marsh, B.B. & Leet, N.G. (1966):
Studies in meat tenderness. 3. The effects of cold shortening on tenderness. J. Food Sci. 31:450-459.

Marsh, B.B., Lochner, J.V., Takahashi, G. & Kragnes, D.D. (1980-81):
Effects of early postmortem pH and temperature on beef tenderness. Meat Sci. 5:479-483.

Marsh, B.B., Ringkob, T.P., Russell, R.L., Swartz, D.R. & Pagel, L.A. (1987):
Effects of early postmortem glycolytic rate on beef tenderness. Meat Sci. 21:241-248.

Marsh, B.B., Woodhams, P.R. & Leet, N.G. (1968):
Studies in meat tenderness. 5. The effects on tenderness of carcass cooling and freezing before the completion of rigor mortis. J. Food Sci. 33:12-18.

McCrae, S.E., Seccombe, C.G., Marsh, B.B. & Carse, W.A. (1971):
Studies in meat tenderness. 9. The tenderness of various lambs muscles in relation to their skeletal restraint and delay before freezing. J. Food Sci. 36:566-570.

Olson, D., Parrish, F.C. & Stromer, M.H. (1976):
Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. J. Food Sci. 41:1036-1041.

Petaja, E., Kukkonen, E. & Puolanne, E. (1985):
Effect of post-mortem temperature on beef tenderness. Meat Sci. 12:145-154.