THE EFFECT OF TEMPERATURE ON THE RIGOR MORTIS DEVELOPMENT OF EXCISED LAMB MUSCLES.

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SUMMARY.

Development of rigor mortis and related parameters, as affected by the temperature of postmortem storage, have been studied in lamb *Longissimus dorsi*. Results confirmed previous reports on the patterns of postmortem variation of pH, lactic acid, ATP, fibre shortening, extensibility and water holding capacity for other animal species. At lowest temperatures (0-4°C) shortening was very intense and occurred at initial pH and ATP concentration; intermediate temperatures (10-20°C) resulted in a weak shortening which occurred at pH 6,1-6,3 and 50% the initial ATP concentration. Howewer it is worth to point out that beginning and end of shortening and extensibility were totally coincident at all studied temperatures. Water holding capacity was not affected either by shortening or by rigor development, and was found to be directly related to pH.

INTRODUCTION.

It has been known for many years that conditioning temperature exerts a dramatic effect on postmortem muscle metabolism (Bate-Smith and Bendall, 1949) . It's generally accepted that low temperatures cause cold shortening to appear (Locker and Hagyard, 1963; Honikel et al., 1983) and this has been directly related to meat thoughening (Marsh and Leet, 1966) or even to changes in water holding capacity (Honikel et al., 1986). On the other hand, some authors found tender meats when carcasses were held at high temperatures either along rigor development (Petaja et al., 1985) or over a short period early postmortem (Lochner et al., 1980; Marsh et al., 1980-81).

However, most of previous reports, except that of Honikel et al. (1983) using beef muscles, dealt with whole carcasses, where temperature decrease of muscles is known to be a slow process. When muscles are excised from the carcass they react further more rapidly to temperature changes and are allowed to undergo unrestrained cold- or rigor-shortening. One can therefore get a closer insight into the mechanisms involved and the subsequent effects of shortening on the loss of extensibility or the water holding capacity of those muscles.

MATERIALS AND METHODS.

Thirty lambs of 9-12 Kg carcass weight were slaughtered by exsanguination after electrical stunning. *Longissimus dorsi* muscles were excised from carcasses within 30 min after slaughter. The muscles were immediately trimmed of visible fat, wrapped in polyethylene bags and brought to internal temperatures

of either 0, 4, 10, 15, 20 and 36°C in a water bath. They were then maintained at those temperatures until rigor mortis was fully developed.

Several bundles of muscle fibres of about 0,3 cm diameter and 5 cm length were excised from whole mucles within an hour postmortem in order to measure extensibility and degree of shortening. Muscle samples were taken at intervals of 1hr or 1hr 30 min during development of rigor mortis in order to measure: pH, R-value, lactate concentration and WHC.

Shortening of fibres.- It was expressed as the percentage of the difference between initial (after bundles excision) and final (rigor onset) lengths related to initial value.

Extensibility of fibres.- Unloaded bundles were measured (unloaded length) at intervals of 1hr or 1hr 30min; then they were hung up and stretched by loading with 5-10g (≈ 100-150 g/cm^2) for a short time and the increase in length was measured. The extensibility was expressed as the percentage of the difference between unloaded and loaded lengths related to unloaded length.

pH of tissue.- About 3 g of muscle were homogenised in 20 ml distilled water for 15 sec using an Ultra-turrax (Janke-Kunkel). The measurement was carried out immediately using a Crison pH-meter and a combined glass electrode.

Lactate determination.- It was determined according to Noll (1974).

R-value.- It was determined by diluting 0,1 ml perchloric extract with 1,9 ml of 0,1 M phosphate buffer pH 6.5 and measuring the ratio of absorption at 250 and 260 nm, according to Honikel and Fischer (1977).

The correspondence between R-values and ATP concentration is shown in table 1, according to the results obtained from Roncalés et al. (1989).

Table 1.- Conversion of R Value into approximate ATP concentration in lamb *Longissimus dorsi* muscles. (According to Roncalés et al., 1989).

R-value	Approximate ATP concentration (mM)	Per cent of initial concentration
0.85	4.90	100
0.9	4.18	85
0.95	3.52	72
1	2.92	60
1.05	2.38	49
1.1	1.89	39
1.15	1.46	30
1.2	1.09	22
1.3	0.51	10
1.4	0.17	3
1.5	0.05	1

Water Holding Capacity.- We have used the press method according to Grau and Hamm (1957).

RESULTS AND DISCUSSION.

Muscle final temperature for each treatment was reached within 3-4 hours postmortem in all cases and it was held constant until the onset of rigor mortis.

Evolution of pH. It is well known that temperature increase causes a rapid fall in pH. Results presented in figure 1 show the effect of temperature on pH changes revealed by time needed to reach final pH, which decreased with increasing temperature: 24 hr at 0°C, 20 hr at 4°C, 17 hr at 10-20°C and 8 hr at 36°C.

The mean pH at the beginning of storage was 6.65 with a standard deviation of 0.19, while ultimate pH varied with prerigor temperature. At low temperatures (0°C) final pH was 5.85, higher than at other studied temperatures; results are in agreement with those reported by Bouton et al. (1973). At 36°C ultimate pH was 5.65, higher than at 4°C, which showed a medium value of 5.57 and 10-20°C, whose pH was 5.48.

Lactate concentration. As shown in figure 2, relationship between lactate concentration and pH in postmortem muscle is linear and independent of temperature of treatment.

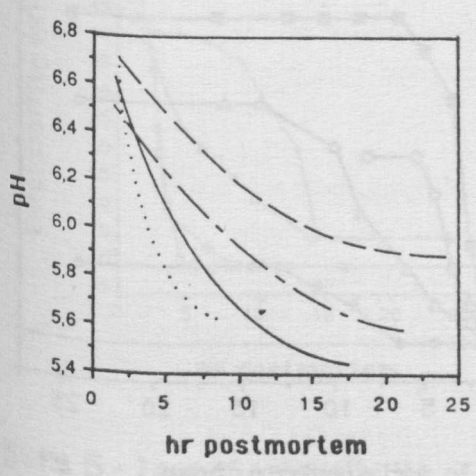

Figure 1.- Changes in pH during post-mortem storage of lamb *Longissimus dorsi* at different temperatures: - - - 0°C — — — 4°C, —— 10-20°C and ····· 36°C.

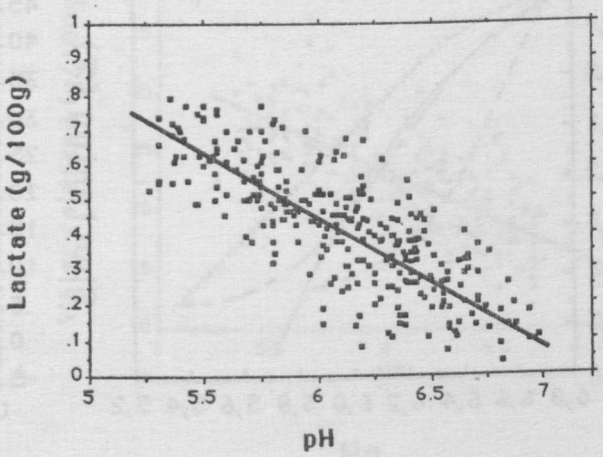

Figure 2.- Relationship between lactate formation and the fall of pH in lamb *Longissimus dorsi* at several postmortem temperatures of storage 0°C, 4°C, 10-20°C and 36°C.

R-value (ATP concentration). R-value is an indirect, rapid, reliable and simple method to determine ATP concentration, highly correlated with the levels of the nucleotides obtained by enzymic analysis (Jolley et al., 1980-81; Roncalés

et al., 1989). Results shown in figure 3 evidentiated the accepted two stage-pattern of ATP depletion (Bendall, 1973), consisting of an initial delay phase (during which ATP depletion is matched by resynthesis) followed by a linear fall in concentration.

Initial R-value is approximately 0.85 (4.90 µmol ATP/g muscle) while final R-value varied with temperature of treatment. At 0°, 4° and 10-20°C its value was 1.40-1.45, but at 36°C final value was 1.55. Explanation for this phenomenon is still not known.

Evolution of R-value at 0°C differed considerably of that at higher temperatures, since it increased at a bigger rate. This appeared to be a consequence of the acceleration of metabolism at low temperatures due to a rapid ATP depletion caused by the great amount of the nucleotide needed for the intense cold shortening to occur.

At pH 6.0 ATP was completely depleted following 0°C treatment, muscles conditioned at 4° and 36°C had about 1 µmol ATP/g muscle, while those held at 10-20°C maintained levels of ATP of about 2 µmol ATP/g muscle. ATP concentration was 0 in all cases when ultimate pH was reached, except for experiments at lowest temperatures (0° and 4°C). It was surprising too that at an ATP concentration of 0 pH still decreased. Apparently, ATP synthesyzed is being immediately hydrolyzed at such a rate that ATP level within the muscle cell remains at a very low non-detectable level (Hamm, 1977).

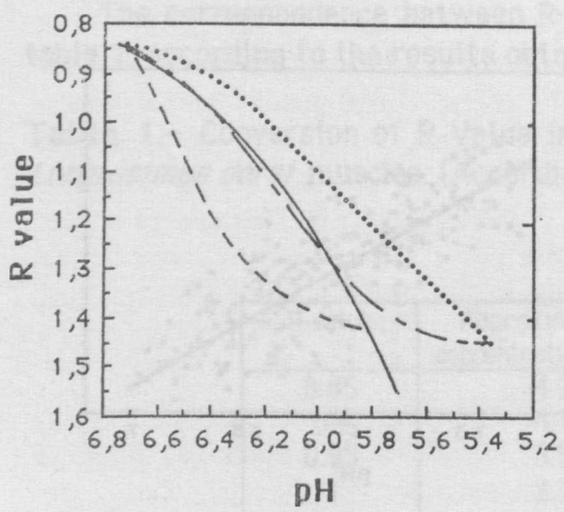

Figure 3.- Changes in R-value related to pH fall in lamb *Longissimus dorsi* muscles stored at various postmortem temperatures:-----0°C,— - - —4°C, ... 10-20°C and —— 36°C.

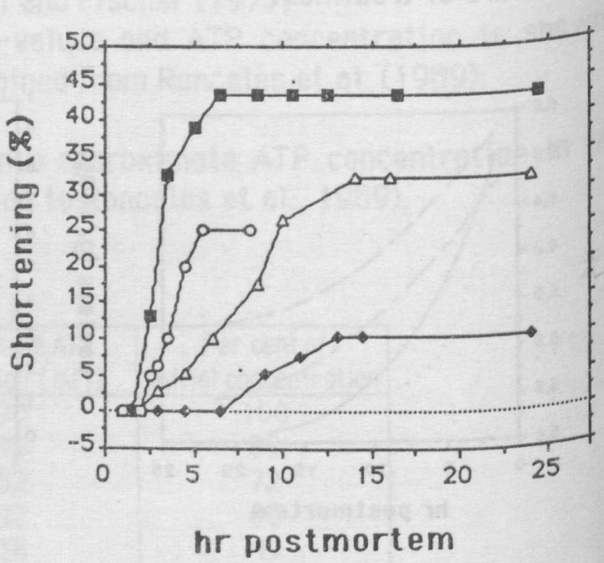

Figure 4.- Shortening of lamb *Longissimus dorsi* during postmortem storage at different temperatures: ■ 0°C △ 4°C, ♦ 10-20°C and ○ 36°C.

Shortening and extensibility of the fibres. As shown in figure 4, fibre shortening was found to occur at every storage temperature; though degree and speed of contraction varied with different temperatures. Experiments performed at 0°C presented the most intense and fastest contraction, reaching about 40% shortening within only 6 hours postmortem. Muscles held at 10-20°C showed the lowest shortening (≈ 10%), and it began at 8 hours postmortem.

Figure 5 is representative of the evolution of extensibility at all studied temperatures. Beginning of extensibility loss defines, according to Bendall (1973), the onset of rigor mortis, being rigor fully established when muscle has totally lost its extensibility. Honikel et al. (1983) completed this definition as the irreversible loss of extensibility of a bundle of muscle cells as a whole. It is noticeable in figure 5 that extensibility is maintained at a high level during approximately 10 hours at 10-20°C, while at 4°C there was a decrease in extensibility until a 12% loss was reached. At high and low temperatures (36º and 0°C) extensibility was already nonexistent by 10 hours postmortem.

Water Holding Capacity. As is shown in figure 6, postmortem fall of water holding capacity in muscles at every studied temperature was directly correlated to changes in pH. Jolley et al. (1980-81) reported the lack of effect of storage temperature on the water holding capacity of muscle; our results agree with their findings, since we demonstrated a linear relationship between pH and water holding capacity for all temperatures of treatment.

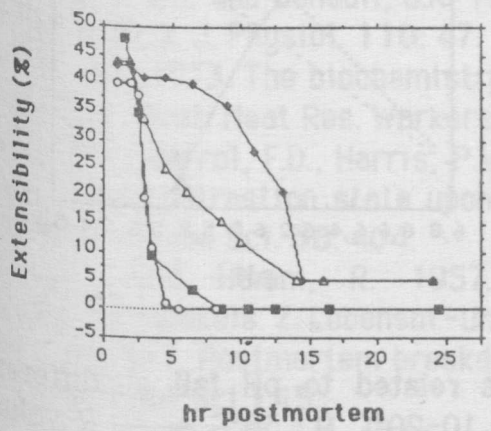

Figure 5.- Loss of extensibility of lamb *Longissimus dorsi* muscles stored post-mortem at various temperatures: ■ 0°C, △ 4°C, ◆ 10-20°C and ○ 36°C.

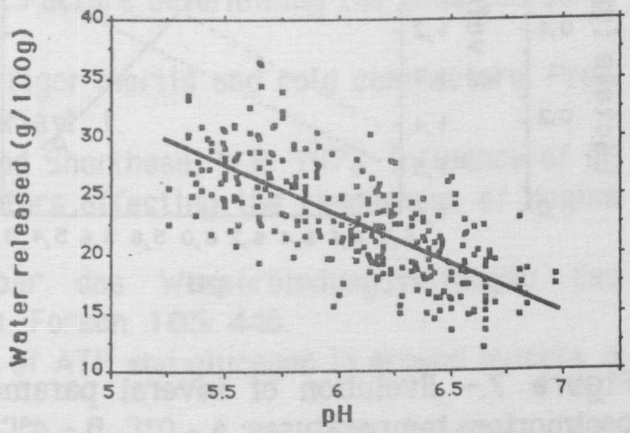

Figure 6.- Changes in water holding capacity, expressed as g water released / 100 g muscle, in relation to the postmortem pH fall in *Longissimus dorsi* muscles stored at different temperatures: 0, 4, 10-20 and 36°C.

Figure 7 summarizes changes associated to rigor mortis development of lamb muscle under different temperatures of treatment used throughout our study. Temperature-dependent differences previously shown for each measured parameter appeared even more evident when plotted together for each temperature.

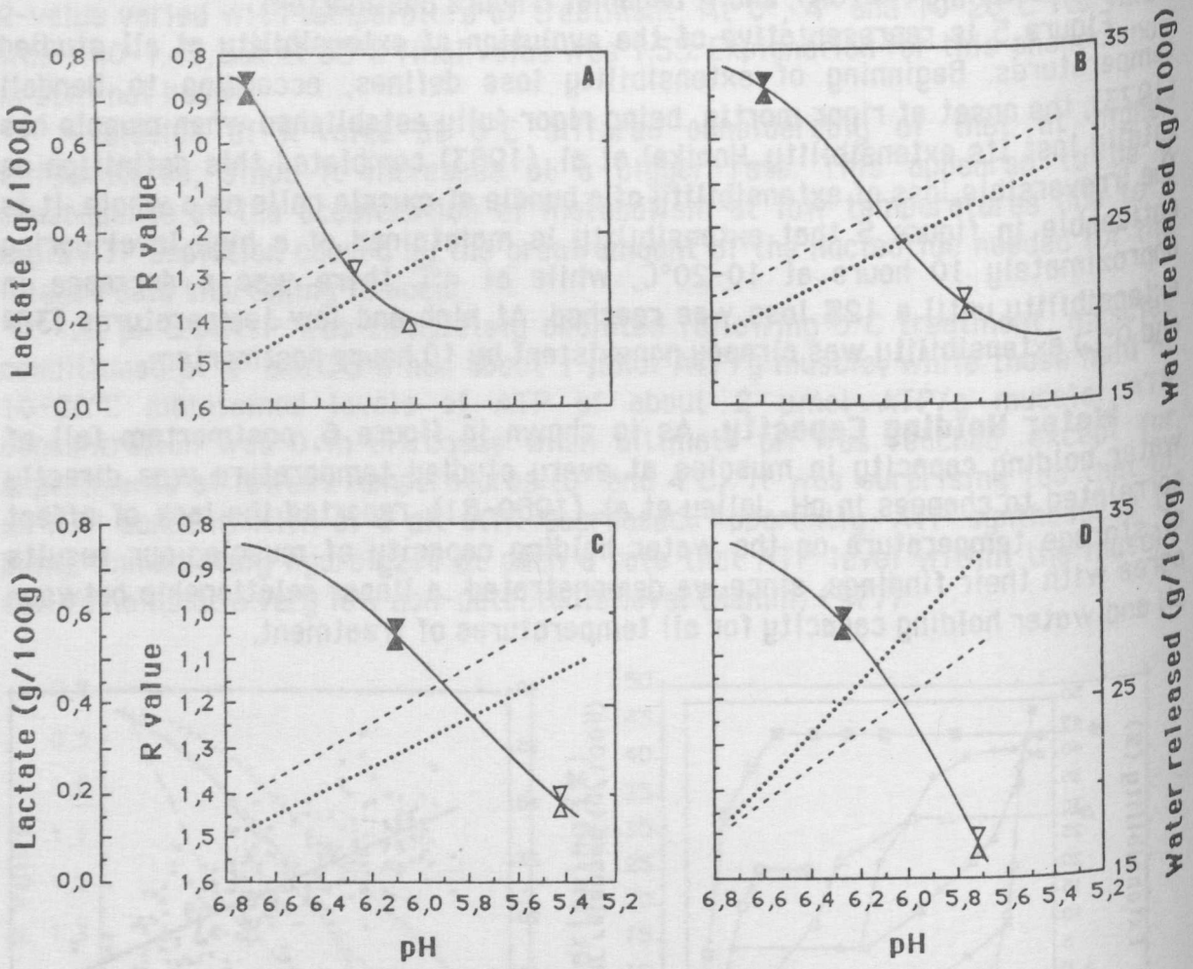

Figure 7.- Evolution of several parameters related to pH fall at different postmortem temperatures: A.- 0°C, B.- 4°C, C.- 10-20°C, D.- 36°C. ——— R-value, ------- Lactate concentration, ... WHC, ▼ beginning of shortening, ▽ end of shortening, ▲ beginning of extensibility loss, △ end of extensibility loss.

A major feature was the fact that patterns of fibre shortening and loss of extensibility were almost totally coincident for all temperatures, since both processes started and concluded at the same pH and ATP concentration. Differences among temperatures were nevertheless apparent. Lowest temperatures (0-4°C) caused immediate shortening to occur, while fibres contained their full ATP concentration and had consequently a high pH; This cold shortening determined a reversible loss of extensibility of fibres, according to Honikel et al. (1983). Both processes concluded before final pH was reached and

while fibres were not completely depleted of ATP. All this suggest that rigor mortis development and loss of extensibility are not at all coincident.

All other temperatures (10-36°C) resulted in a delayed beginning of shortening and loss of extensibility, not apparent until pH reached a value of 6.1-6.3 and ATP concentration fell to about 2.5 µmol/g. Extensibility disappeared and shortening ceased only when final pH was reached and fibres became depleted of ATP. Rigor development can be then effectively monitored by following either shortening or extensibility loss, what makes much easier its measure.

Figure 7 also revealed the close relationship between either lactate formation or water holding capacity and muscle pH, showing a linear increase or decrease respectively during postmortem storage, highly correlated to pH fall. The evolution of both parameters was non-dependent on changes associated with shortening or rigor onset, in agreement with results reported for beef by Honikel et al. (1981a,b). They appeared to be non-dependent too on temperature of treatment, with the sole exception of the rate of decrease of water holding capacity when muscles were held at 36°C, indicating a likely denaturation of myofibrillar proteins.

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