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The influence of temperature on the course of rigor and aging in two beef muscles.

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Introduction

Tenderness is generally judged to be the most important quality attribute of whole meat. Variations in tenderness can be related to the quality or maturity of the connective tissue in the meat, and/or it can arise from the myofibrillar toughness. In this papar our attention will mainly be directed towards myofibrillar toughness. Myofibrillar toughness is very much influenced by the handling of the carcass after death, i.e. the conditions prevailing pre and post-rigor. The temperature during this handling exerts a strong influence on the course of rigor and aging and thereby on myofibrillar toughness.
Locker and Hagyard (1963) found that shortening in rigor showed a minimum of about 10% in the region of 15-20°C, i.e. there exists a rigor shortening region from 20 to 40°C (up to 30% shortening) and a cold shortening region from about 14 to 0°C (up to 50% shortening). Marsh and Leet (1966) demonstrated that when muscles cold shorten to approximately 20% of the excesid resting length, no appreciable reduction in tenderness ensues. Beyond 20% shortening, tenderness was shown to decrease very rapidly to a peak value (maximum toughness) at approximately 40% shortening. Evidently, according to these investigations, neither a higher nor a lower temperature than 15°C pre-rigor is favourable with regard to meat tenderness.
The matter is complicated, however, by the fact that if meat is kept warm after slaughter, membrane-bound lysosomal enzymes are more easily released. Wu et al. (1981) have shown for example that for individual muscles a period (12h) of conditioning at 37°C promotes a rapid and substantial release of lysosomal hydrolases. Lochner et al. (1980) have postulated that the tenderness of meat is markedly affected by higher temperatures (27-40°C) 2-4h post mortem. Locker and Daines (1975) have also shown that muscles which have been allowed to shorten during rigor at 37°C are in some cases actually more tender than unshortened ones.
These contradictory results found in the literature illustrate the need for an investigation to try to clarify which event (i.e. the shortening or the proteolytic degradation of the myofibrillar structure) has most impact on beef muscle tenderness. A possible way to elucidate this is to study the course of rigor and aging of beef muscle at the two different rigor temperatures of 15 and 37°C, respectively. According to the literature the former temperature will induce the least shortening and therefore the most tender meat and the latter temperature will give rise to the most proteolytic activity and thereby the most reduced toughness during aging.
The course of post mortem changes was registered by the isometric tension and by the shortening of unrestrained muscle strips. The events during aging post rigor (+4°C) were followed by measuring the myofibril fragmentation index and by evaluating the sensory properties of the cooked meat. Two beef muscles were studied, M. biceps femoris (BF) and M. semitendinosus (ST), representing muscles of different fibre type composition.

Materials and Methods

Sample handling

M. biceps femoris (BF) and M. semitendinosus (ST) were sampled from young bulls approximately 15 min after slaughter. For the isometric tension and shortening experiments strips at 1h post mortem approximately 35 mm long and 20-30 mm^2 in cross-sectional area were carefully cut from the muscle sample parallel to the fibre axis. The whole muscle was vacuum-packed in a plastic bag and stored at 15 and 35°C, respectively, until completion of rigor, which was determined by the isometric tension measurements. Thereafter, the muscle was stored at +4°C.

Isometric tension and muscle shortening

The excised strips were blotted dry and immediately weighed. This weight was used together with the measured length of the strips and the density of muscle to calculate the cross-sectional area of the strips. The muscle strip was then covered with paraffin-oil to provide an anaerobic environment and to minimize dehydration.
To minimize slippage of fibres, the ends of the strips were glued with a cyanoacrylate glue (loctite superglue) onto aluminium discs, which were screwed onto the isometric tension apparatus. For recording the isometric tension a force displacement transducer SWEMA SG3 was used. It is designed for the measurements of small forces at very limited displacement (± 2.92 µm/N).
The initial tension, the force of gravity, was noted and the latter was substracted from all subsequent values of isometric tension. The tension was registered every 15th minute by a microcomputer Luxor ABC 80 (a Z 80A processor based computer).
Measurements were carried out in a jacketed chamber, where the temperature, 15 and 37°C, was regulated by circulating water from a water-bath.
Strips for shortening experiments were glued using the cyanoacrylate glue (loctite superglue) at one end to a screw-clamp and allowed to hang freely at the other. The strips were held at a constant temperature of 15 and 37°C, respectively. Muscle shortening was followed every 30th minute using a vernier calliper.

Myofibril fragmentation

1h post mortem a piece of the muscle sample approximately 2x5x15 cm was glued onto a screw clamp at both ends without being stretched. It was stored anaerobically at 15 and 37°C, respectively, until completion of rigor (as determined by isometric tension measurements). Thereafter, the muscle piece was freed from the screw clamp and vacuum-packed and stored at +4°C up to 16 days post mortem.
Myofibrils from the muscle pieces were isolated at intervals up to 16 days post mortem according to the procedure given by Olsen et al. (1976). The homogenizer used in the procedure was a Sorvall omni-mixer working at 11,000 rpm for 60s.
A suspension with a protein content of 0.5±0.05 mg/ml was made from the sediment of the myofibrils from the last centrifugation. The myofibrillar fragmentation index (MFI) was determined as absorbance value at 540 nm of the myofibril suspension (Olsen et al., 1976) with a standard error of at the most ±0.02 in absorbance.

Sensory evaluation

Sensory analyses were carried out 4, 8 and 12 days post mortem.
1.5 cm thick slices were cut from the whole muscle on every occasion and put into heat resistant plastic bags (Lovatec-OTE). The evacuated bags were then put into a water-bath at 80°C and cooked for 60 minutes.

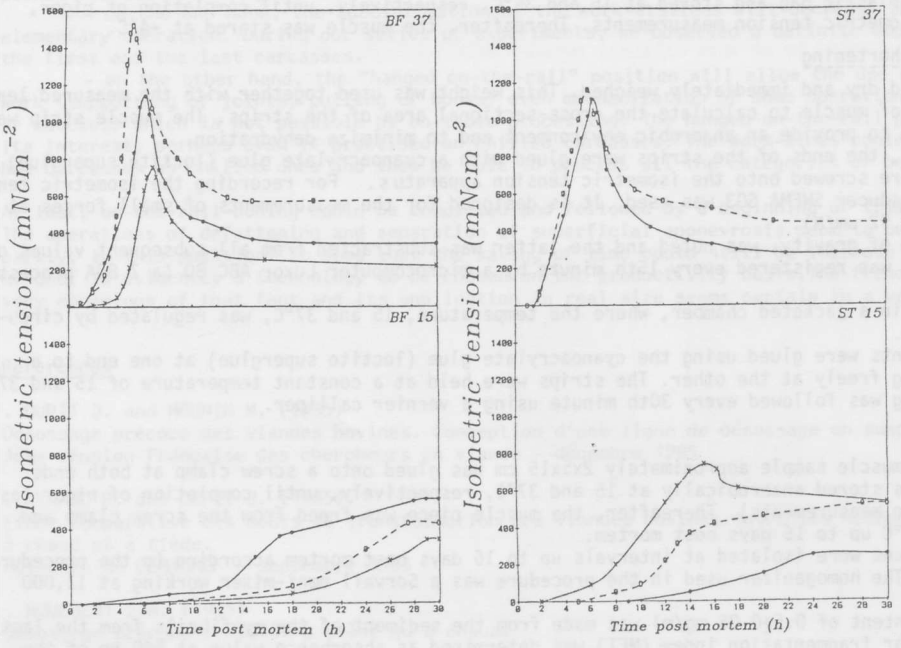
A trained expert panel consisting of 10 people assessed sample pieces (4x3 cm) which were served immediately after being cooked. The sensory properties of the cooked muscles were evaluated using a profile which included the attributes tenderness (1=none, 9=very large), jiuciness (1=none, 9=very large), chewing time (1=very short, 9=very long), chewing residual (1=none, 9=very large).

Results and Discussion

Isometric tension and muscle shortening

In Figure 1, the time-course of rigor development, as followed by isometric tension, can be seen for M. biceps femoris and M. semitendinosus at 15 and 37°C, respectively.
It can be noted that the curves are characterized by a delay period before any significant development of tension commences. This delay period is very much shorter at 37°C than at 15°C, for both the muscles studied. The delay period is then followed by a relatively rapid increase in tension reaching a maximum, whereafter a decline in tension occurs and finally a plateau value being achieved. The speed at which maximum tension is developed and the maximum tension is obtained is also very dependent on temperature, being larger at 37 than at 15°C. This temperature dependence on rigor development has been observed by a number of researchers since the work of Bate-Smith (1939), but, regarding studies on isometric tension in particular ,the work of Busch et al. (1972) and Nuss and Wolfe (1980-81) should be mentioned.
Each curve represents a single muscle from a single animal and, as can be seen from the figures there is a variability in results. Also, Busch et al. (1972) noted that there could be considerable variation amongst different animals in the amount of isometric tension development and the time after death at which isometric tension development occurs.

Figure 1. Isometric tension as a function of time post mortem at 15 and 37°C for M. biceps femoris (BF) and for M. semitendinosus (ST). Each curve represents a single experiment.

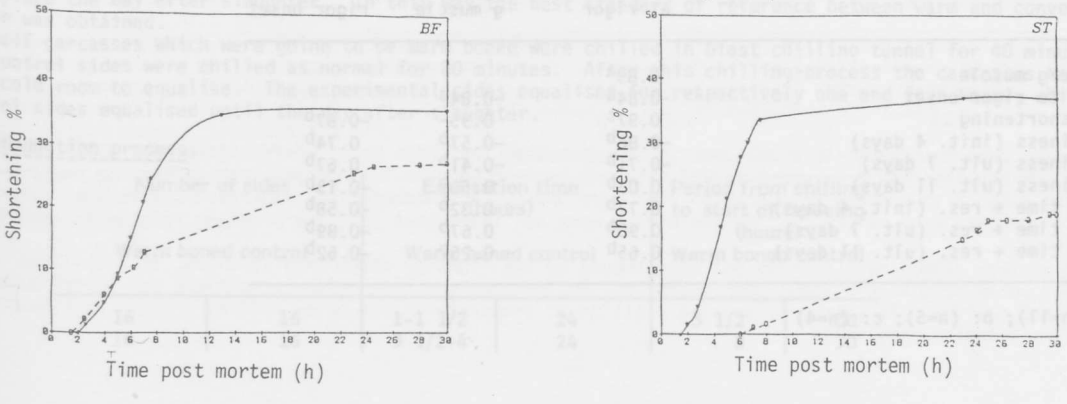


The shortening of unrestrained strips of muscle was followed as a function of time post mortem for four of the samples (2 muscles, 2 temperatures). The results are shown in Figure 2 for M. biceps femoris and M. semitendinosus at 15 and 37°C, respectively.
For both muscles, shortening is more pronounced at 37°C than at 15°C, which agrees well with other studies on bovine muscle (Locker and Hagyard, 1963 and Cassens and Newbold, 1967). Our results, however, show a higher degree

of shortening, being about 35% for both muscles at 37°C and 25% for BF at 15°C and about 18% for ST at 15°C. Locker and Hagyard (1963) showed a minimum of shortening at 15°C to be about 10%, whereas Cassens & Newbold (1967) arrived at so low an amount of shortening as 5% at 15°C. These differences in shortening might be attributed to a different beef muscle being studied in the other investigations (beef sternomandibularis) and by different procedures of measurements (cyclically loaded strips in the case of Cassens and Newbold, 1967).

It is interesting to note the remarkable difference between the time-course of shortening and the time-course of isometric tension after maximum shortening and tension, respectively, ara attained. This is apparent when Figures 1 and 2 are compared. For the isometric tension at 37°C, there is a declining part which levels out, whereas this declining part is absent in the shortening experiment. Busch et al. (1972) have also noted this phenomen and assign the declining part of the isometric tension curve to a so called resolution of rigor. This dissipation of rigidity was supposed to be caused by proteolytic enzymes. They did not succeed, however, in showing that pH and extracellular proteases are involved in rigor resolution. We will instead suggest that the declining part of the isometric tension curve is an artefact from these types of measurement and that resolution of rigor does not occur. We can see from Figure 1 that when rigor development is slow and maximum tension is low, like it is at 15°C, no such maxima in tension, as at 37°C occurs. Therefore, we suggest that the loss in tension after maximum tension being achieved can be due to local fractures within the muscle piece, which are formed by the high tension and the high rate of tension build up at 37°C. To further confirm this statement a muscle piece from M. semitendinosus was attached to the isometric tension apparatus and stretched to only 87% of this length. Temperature was held at 37°C and tension was registered. The same time course of rigor as for the fully stretched samples was recorded but only a maximum tension of 500 $mNcm^{-2}$, and no decline in tension was observed.

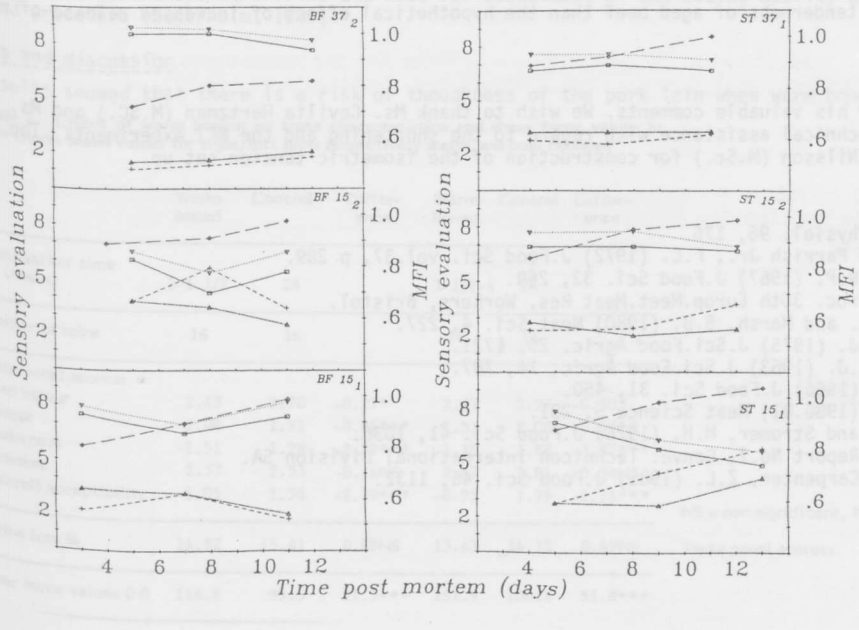
Figure 2. Percentage shortening as a function of time post mortem at (--0--) and 37°C (-x-) for M. biceps femoris (BF) and M. semitendinosus (ST). Each curve represents a single experiment.



Myofibril fragmentation and sensory evaluation

Six samples run for myofibrillar fragmentation index (MFI) and sensory analyses as a function of 2 to 13 days post mortem storage at 4°C are presented in Figure 3. The temperatures given in the figure denote the temperature until rigor completion, BF and ST being the designations given to the two types of muscle studied. The sensory scale varied from 1 to 9 and a standard error of 0.3 to 1.5 was obtained.

Figure 3. Sensory evaluation (tenderness (+---+), juiciness (Δ---Δ), chewing time (▽-▽), residual (□—□) and myofibril fragmentation index (MFI◇--◇) as a function of time of aging for M. biceps femoris and M. semitendinosus, when rigor is entered at 15 and 37°C, respectively. Each curve represents a single experiment.



As can be seen from the figure, MFI increase and sometimes levels off during post mortem storage for all six samples. This behaviour has also been the experience of Olsen et al. (1976) for bovine longissimus, semitendinosus and psoas major muscles and of Fjelkner-Modig (1984) for bovine and porcine longissimus dorsi.
From Figure 3 it can further be evaluated that tenderness increases only slightly as a function of time post mortem, for the BF 37 and ST 37 samples. For the ST 15 samples, the tenderness improvement is greater and for the BF 15 sample, there is a maximum in tenderness at 7 days post mortem.
Juiciness follows tenderness relatively closely for the samples that developed rigor at 37°C, whereas this close agreement between the two sensory attributes is not so pronounced for the samples that passed into rigor at 15°C. Chewing time and chewing residual are clearly related and almost inversely proportional to tenderness.

The relationships between different parameters of the course of rigor and aging

To give a general idea of the results obtained from the course of rigor and aging we have collected in Table 1 the interrelationships expressed as correlation coefficients between the different parameters of rigor and aging. According to Table 1, the maximum isometric tension, the force per g muscle and the degree of the larger the maximum isometric tension and the higher the degree of shortening of the unrestrained muscle will be. Time to rigor onset is also well correlated negatively to maximum tension developed (r=-0.84). This suggests that the time to rigor onset should be as long as possible as in order to obtain minimum shortening.

Table 1. The relationships between different measurements of the course of rigor and tenderness and juiciness given as correlation coefficients of linear regression analyses.

	Max tension at rigor	Force g muscle	Time to rigor onset
Force/g muscle	0.89[a]		
Time to rigor onset	-0.84[a]	-0.84[a]	
Max shortening	0.97[c]	0.95[c]	-0.87[b]
Juiciness (init. 4 days)	-0.86[b]	-0.57[b]	0.74[b]
Juiciness (ult. 7 days)	-0.71[b]	-0.41[b]	0.67[b]
Juiciness (ult. 11 days)	0.03[b]	0.50[b]	-0.13[b]
Chew time + res. (init. 4 days)	0.77[b]	0.32[b]	-0.58[b]
Chew time + res. (ult. 7 days)	0.90[b]	0.67[b]	-0.89[b]
Chew time + res. (ult. 11 days)	0.65[b]	0.25[b]	-0.62[b]

a: (n=11); b: (n=5); c: (n=4)

In Table 1, the sensory attributes juiciness and chewing time plus residual after 4, 7 and 11 days conditioning have been correlated to the different parameters characterising the course of rigor. The correlations given in Table 1 indicate that the slower the rigor development, the less isometric tension, the less shortening of muscle and the more tender the meat will be. It is, according to Marsh and Leet (1966) detrimental to meat tenderness if shortening during rigor is between 20 to 40%, the more shortened - the tougher. The shortening of the muscles increases to 35-37% at 37°, which also results in tougher meat than at 15°C, where the shortening is less (18% for ST and 26% for BF). The tenderising effect can, when the muscle is shortened to a lesser degree, even be so substantial that a maximum in tenderness during a fortnight's storage can be obtained. This is also reflected in Table 1 where lower correlation coefficients between the parameters governing the course of rigor and the tenderness parameters for the samples stored for 11 days compared to those stored for 7 days are obtained. Thus, our conclusion from this investigation is that the beneficial effect of slow rigor development and minimum contraction at 15° is more important for tenderness of aged beef than the hypothetical effect of increased release of proteolytic enzymes at 37°.

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