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# Effect of Pre-rigor Stretch and Various Constant Temperatures on the Rate of Post-mortem pH Fall, Rigor Mortis and Some Quality Traits of Excised Porcine *Biceps Femoris* Muscle Strips

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## ABSTRACT

Porcine biceps femoris strips of 10 cm original length were stretched by 50% and fixed within 1 hr post mortem then subjected to temperatures of  $4^{\circ}$ ,  $15^{\circ}$  or  $36^{\circ}C$ until they attained their ultimate pH. Unrestrained control muscle strips, which were left to shorten freely, were similarly treated. Post-mortem metabolism (pH, Rvalue) and shortening were recorded; thereafter ultimate meat quality traits (pH, lightness, extraction and swelling of myofibrils) were determined. The rate of pH fall at  $36^{\circ}C$ , as well as ATP breakdown at 36 and  $4^{\circ}C$ , were significantly reduced by pre-rigor stretch. The relationship between R-value and pH indicated cold shortening at  $4^{\circ}C$ . Myofibrils isolated from pre-rigor stretched muscle strips kept at  $36^{\circ}C$  showed the most severe reduction of hydration capacity, while paleness remained below extreme values. However, pre-rigor stretched myofibrils — when stored at  $4^{\circ}C$  — proved to be superior to shortened ones in their extractability and swelling.

## INTRODUCTION

According to recent knowledge about pork meat quality, ultimate meat quality is not consistently related to  $pH_1$  and other early post-mortem data (Eikelenboom, 1987; Eikelenboom & Nanni Costa, 1988; Warris & Brown, 1987; Kauffman *et al.*, 1993), and the interrelationship between meat quality traits is quite variable (Eikelenboom & Nanni Costa, 1988). These conclusions have promoted research for further post-mortem phenomena which would provide additional explanations for the variation of meat quality.

In general, the influence of temperature, as a major post-mortem factor, has been thoroughly investigated. The temperature dependence of rigor shortening has long been recognised (Locker & Hagyard, 1963). Post-mortem energy metabolism and related muscle contraction, which was marked at low ( $< 10^{\circ}$ C) and high ( $> 30^{\circ}$ C) temperature, were studied at different constant temperatures of the onset of rigor mortis in beef (Jolley et al., 1981; Honikel et al., 1983; Hertzman et al., 1993) and lamb (Beltrán et al., 1993), as well as in pork (Bendall, 1975; Fernandez & Tornberg, 1993).

The interaction of temperature and pH to cause PSE pork, in the case of fast glycolysis, is well established. The practical use of rapid chilling to diminish the pale and exudative character of fast glycolysing pork was suggested by Borchert & Briskey (1964) and Honikel (1986) and the risk of cold shortening in causing higher drip was emphasized in respect of slowly glycolysing pork by Honikel & Reagan (1987).

Considering the known effects of high temperature and low pH, one can expect both considerable shortening and protein denaturation. In contrast to this, Honikel & Kim (1986), Eikelenboom & Nanni Costa (1988) and Irving *et al.* (1989) reported on greater sarcomere length of PSE muscle than that of other quality types. Larsson & Tornberg (1988) observed that PSE meat could have both the longest sarcomeres and also the shortest. Honikel & Kim (1986) concluded that the greater sarcomere length of PSE pork might be associated with the loss of contractability at the onset of rigor mortis. This assumption was supported by the result of Sung *et al.* (1976) who observed reduced solubility of contractile proteins together with reduced contractability of myofibrils in the loins of low pH<sub>1</sub>. Discussing the results of Irving *et al.* (1989), who found a higher proportion of free crossbridges of myosin in PSE pork samples, Bendall & Swatland (1988) supposed that restoration of the pre-rigor state might be related to softness.

It can also be supposed however, in considering the greater sarcomere length of PSE pork, that contraction might be physically inhibited by skeletal restraint, especially in major muscles of the pelvic limb, when ATP depletion occurs very early postmortem (PSS syndrome), prior to lifting the carcass to the rail.

Skeletal restraint would be expected to vary not only because of carcass position, but also because of muscular cramps evoked by stunning. In fact, marked variation of sarcomere length was reported by Larsson & Tornberg (1988) and Vada-Kovács (1992) within the major pork quality categories.

Unfortunately, studies of the extreme skeletal restraint caused by pelvic suspension of the pig carcass, whereby some muscles are kept stretched, has been focused mainly on the elimination of toughness caused by ultra rapid chilling (Møller & Vestergaard, 1986; Møller *et al.*, 1987; Smulders *et al.*, 1992) and less attention has been given to the importance of the stretching of muscle in relation to the overall quality variation in pork, except for low drip loss. Pelvic suspension has been applied also in combination with electrical stimulation (Dransfield *et al.*, 1991; Taylor *et al.*, 1992) which is known to evoke fast glycolysis. Denaturation of myosin, responsible for most of the PSE symptoms (Offer, 1991), was evident at low pH and high post-mortem temperature (Penny, 1967; Sung *et al.*, 1976; Stabursvik *et al.*, 1984; Honikel & Kim, 1986). However the degree of denaturation has been less investigated in relation to stretched pre-rigor muscle, despite the postulated protective role of rigor bonds (Penny, 1967; Offer, 1991), which would depend on the overlapped area of myofilaments.

For a better understanding of the variation of muscle length and pork quality through of multiple interactions with temperature and post-mortem time, a more complex study is to be carried out simulating early post-mortem variation of factors investigated. Homogeneous experimental material is also important, otherwise high sampling error would overcome the effect of the factors investigated. Since, hitherto, research has not been focused directly on the course of rigor mortis in pork of different pre-rigor muscle length in relation to temperature, the object of the present study was to evaluate the rate of energy breakdown, and associated water-holding capacity, in terms of the solubility of myofibrillar protein and of swelling as a function of pre-rigor stretch and temperature.

## MATERIALS AND METHODS

## Preparation of muscle strips

A female pig of 120 kg live weight (Landrace breed) was electrically stunned by 90 V for 10 s and immediately bled. *Biceps femoris* muscles were excised from left and right sides of the carcass within 15 min post-stunning. Central section of muscles were freed from fat and connective tissue, and divided into a total of 24 muscle strips by cutting along the fibers. Muscle strips of approx. 30 g, 5–7 mm thickness and 10 cm length were randomly assigned to two groups. One of these was subjected to a pre-rigor stretch of 50% of their original length (the muscle length after removal from the carcass). The other group served as unrestrained controls. The restraint of the stretched strips was carried out by fixing both ends to graded plastic holders using hosecock clamps.

## **Temperature treatment**

After sealing the individual muscle strips into plastic bags, both stretched and control groups were further divided to three subgroups, each containing four muscle strips representing temperature treatments at  $4^{\circ}$ ,  $15^{\circ}$  and  $36^{\circ}$ C in a water bath (GRADIPLATE W10 incubator, Biodata Oy, Finland). Temperature treatments continued until all samples attained their ultimate pH at the chosen temperature, they were then transferred to a refrigerator of  $4^{\circ}$ C.

## Sampling for pH and *R*-value

pH and R-value were determined at intervals 1, 1.5, 2.5, 3.5, 4.5, 6.5 and 9 h after start of incubation using a total of four replicates per each of the six experimental groups. Muscle bundles were separated from randomly selected muscle strips each weighing 3–4 g and divided into subsamples. Duplicate subsamples were immediately used for measuring both pH and R-value in order to determine the end point of temperature treatment according to the ultimate pH. The remaining subsamples were frozen in liquid nitrogen and stored at  $-80^{\circ}$ C until determination of further replicates for pH and R-value.

## pH measurement

Preliminary pH was measured as early as 15 min *post mortem*, in order to control pre-rigor status, using a probe electrode (Ross combination electrode, Typ.8163SC) attached to Knick-Portamess pH-meter. pH measurements were performed immediately before and during temperature treatment as well as on the day after slaughter according to Bendall (1975), when 5 mM iodoacetate-

150 mM KCl, and a 1:10 muscle-solution ratio, were used. pH of the gently homogenized muscle samples (ULTRA TURRAX, Janke-Kunkel, Germany) was determined by Orion Model SA720 pH-meter and combined glass electrode.

## R-value

*R*-value was determined according to Honikel & Fischer (1977) both initially and during thermal treatment.

## Shortening of muscle strips

When sampling for pH and *R*-values during thermal treatment, the length of unrestrained muscle strips was also measured. Shortening was expressed as percentage of the original muscle length.

## Lightness of muscle

At 24 h post mortem lightness  $(L^*)$  was measured by a Minolta Chromameter CR100 on the wiped surface of the muscle strips which had undergone the various temperature treatments. Because of the small thickness of the muscle strips, five sections of each were layered during measuring. Several measurements were taken at different points.

## Preparation, extraction and swelling of myofibrils

A total of six batches of myofibrils were prepared using the muscle strips, which were measured for lightness at 24 h *post mortem* then frozen and stored for 4 days at  $-80^{\circ}$ C.

Fifteen grams of roughly minced muscle was homogenized by ULTRA-TUR-RAX using 5 vol of solution containing 100 mM KCl, 2 mM MgCl<sub>2</sub>, 1 mM EGTA and 10 mM phosphate buffer pH 7.0 (Offer & Knight, 1988). At the start of mincing only 3 vol of solution were added to the meat. The mix was homogenized three times at half-speed for 15 s, then adjusted to the final volume and homogenized again for 30 s. The homogenate was filtered through cheese cloth then centrifuged at 1000 g for 10 min. The pellet was washed four times with the solution used for homogenization. All steps in the preparation were carried out in ice-cooled media. Myofibril preparations were stored at 1°C as pellets and used for determination of extractable protein and swelling within 2 days. Duplicate samples of 1.5-2.0 g were gently stirred with 5 vol of an ice-cold solution of 1.2 M NaCl (giving 1.0 M NaCl as final concentration) for 15 min and sampled immediately or allowed to stand for a further day before sampling for the determination of the total protein content of the myofibril suspension. The myofibril suspension was then centrifuged at 20000 g for 20 min and the supernatant sampled for the determination of extractable protein (which was expressed as percentage of total protein). The protein content was determined by the Biuret method (Gornall et al., 1949), standardized to bovine serum albumin. The swelling of myofibrils was calculated by using the weight of pellet and the total protein content of myofibril suspension. Swelling was expressed as mg extraction solution bound per mg protein.

#### Statistics

Data on pH and R-values were subjected to analysis of variance according to a balanced three-way factorial design. This involved the effect of stretch (two levels), temperature (three levels) and post-mortem storage time (six levels), using four replicates for each treatment combination. Similarly, a balanced three-way factorial design was applied for extractable protein and swelling in 1.0 M NaCl when effect of stretch (two levels), temperature (three levels) and duration of extraction (two levels) were analysed using two replicates for each treatment combination. Means of  $pH_{ult}$  and  $L^*$  values were compared by Student's *t*-test.

#### RESULTS

#### Post-mortem pH decline

At the time of excision of the *biceps femoris* muscle (15 min *post mortem*) pHs of  $6.45 \pm 0.13$  (n=4) and  $6.48 \pm 0.15$  (n=4) were recorded in the right and the left muscles, respectively. The pH level of muscles slightly decreased during the subsequent 35 min when muscle strips were cooled to  $15-20^{\circ}$ C. pH values of  $6.38 \pm 0.13$  (unrestrained, n=4) and of  $6.39 \pm 0.09$  (stretched, n=4) were found at 50 min *post mortem*, immediately before starting the temperature treatments. The pronounced rapid pH fall shortly after killing can be explained by acceleration of muscle glycolysis evoked by electrical stunning (Troeger & Woltersdorf, 1989).

Analysis of variance (Table 1) showed significant main effects of temperature (p < 0.001) and of post-mortem storage time over 9 h (p < 0.001) as well as their significant interaction (p < 0.01). Also, interaction of stretch × temperature was found to be significant (p < 0.05). Figure 1 indicates the retarding effect of prerigor stretch upon the glycolysis of muscle bundles held at 36°C until attaining their ultimate pH. A slower pH fall occurred initially in pre-rigor stretched muscle strips. The ultimate pH was attained within 3.5 and 9 h of storage at 36° and 15°C, respectively, whilst the pH was still above 5.8 after 9 h of storage at 4°C

TABLE 1

Least-square Analysis of Post-mortem pH and *R*-value of Porcine *Biceps Femoris* Muscle Strips Either Stretched Pre-rigor or Left Unrestrained Then Subjected to Different Rigor Temperatures

Source of variance	DF	рН	R-value
Pre-rigor stretch (P)	1	NS	<i>p</i> < 0.05
Temperature (T)	2	<i>p</i> < 0.001	p < 0.001
Storage time (S)	5	p < 0.001	p < 0.001
$P \times T$	2	p < 0.05	p < 0.01
$\mathbf{P} \times \mathbf{S}$	5	NS	NS
$\mathbf{T} \times \mathbf{S}$	10	p < 0.01	p < 0.001
$P \times T \times S$	10	NS	p < 0.01
RSD	108	0.13	0.04

RSD = residual standard deviation.



**Fig. 1.** Time-course of post-mortem pH decline of porcine *biceps femoris* muscle strips as influenced by pre-rigor stretch and rigor temperature ( $\bigcirc = 36^{\circ}$ C;  $\triangle = 15^{\circ}$ C;  $\blacksquare = 4^{\circ}$ C; - = unrestrained; ... = stretched). Each point represents the mean of four replicates. \* Means significantly differ between stretched and unrestrained samples (p < 0.05).

Temperature (° $C$ )	Stretched	Unrestrained
4	5.50 <sup>a</sup>	5 56 <sup>b</sup>
15	5.50 <sup>a</sup>	5.55 <sup>b</sup>
36	5.52 <sup>a</sup>	5.57 <sup>b</sup>

 
 TABLE 2

 Ultimate pH of Porcine Biceps Femoris Muscle Strips as Influenced by Pre-rigor Stretch and Rigor Temperature

<sup>a,b</sup> Means of the same superscript are not significantly differ within the same rows (p < 0.05) (Student's *t*-test) Each value represents the mean of four replicate measurements.

(Fig. 1). The ultimate pH measured at 24 h *post mortem* appeared to be significantly affected by pre-rigor stretch, causing slightly lower pH values (Table 2), which might be attributed to altered buffering capacity.

## Post-mortem breakdown of ATP

Analysis of variance (Table 1) showed that the breakdown of ATP was significantly affected by pre-rigor stretch (p < 0.05). *R*-values were also significantly influenced by temperature (p < 0.001) and post-mortem storage time (p < 0.001). The interaction of stretch × temperature (p < 0.01) and of temperature × storage time (p < 0.001), as well as triple interaction of all factors, were found to be significant (p < 0.01). In Fig. 2, a delay of ATP-depletion by pre-rigor stretch was observed at high temperature incubation during the initial few hours and also at low temperature after 6.5 h of incubation, as compared to control bundles. Although the mechanical criteria of rigor mortis were not tested, it can be concluded from these results, that pre-rigor muscle stretch contributes to the longer prevalence of pre-rigor status both at low and high temperature.

## Relationship between R-value and pH

A linear regression was calculated using the pooled data of stretched and control data sets because no difference was found between curves calculated separately for stretched and control data within the two temperature groups investigated (15–36°C and 4°C). Being independent of *R*-values, pH values above 6.3 were omitted when the linear regression was calculated. Figure 3 shows a definitely slower pH decline at 4°C as compared to 15–36°C temperature, which resulted in higher pH of approx. 0.1 pH unit at the level of 1.25 *R*-value.

## Shortening

Unrestrained muscle strips stored either at low or high temperature exhibited considerable rapid shortening. Total shortening observed at 24 h *post mortem* was 29.3% (4°C), 15.86% (15°C) and 26.86% (36°C) (Fig. 4).

In Fig. 5 the extent of shortening observed at different temperatures *post mortem* are plotted against *R*-values. Marked shortening occurred at  $4^{\circ}$ C but was not accompanied by great increase of *R*-value, which suggests cold-shortening at this



**Fig. 2.** Time-course of ATP-breakdown of porcine *biceps femoris* muscle strips as influenced by pre-rigor stretch and rigor temperature ( $\bigcirc = 36^{\circ}$ C;  $\triangle = 15^{\circ}$ C;  $\blacksquare = 4^{\circ}$ C;  $\_=$  unrestrained; ...= stretched). Each point represents the mean of four replicates. \* Means significantly differ between stretched and unrestrained samples (p < 0.05).



Fig. 3. Relationship between pH and *R*-value at different rigor temperatures ( $= 36^{\circ}C$ ,  $= 15^{\circ}C$ ,  $= 4^{\circ}C$  stretched;  $\bigcirc = 36^{\circ}C$ ,  $\triangle = 15^{\circ}C$ ,  $\square = 4^{\circ}C$  unrestrained). Each point represents the mean of four replicates.



Fig. 4. Time-course of shortening of porcine *biceps femoris* muscle strips at different rigor temperatures (● = 36°C; ▲ = 15°C; ■ = 4°C). Each point represents the mean of four measurements taken on random strips.



Fig. 5. Relationship between shortening and *R*-values at different rigor temperatures  $(\bullet = 36^{\circ}C; \blacktriangle = 15^{\circ}C, \blacksquare = 4^{\circ}C)$ . Each point represents the mean of four replicates.

temperature. In contrast, at 15 and 36°C the start of rapid shortening coincided with a high *R*-value reflecting rigor shortening.

#### Lightness

Lightness ( $L^*$ ) was not influenced either by stretch or by thermal treatment in the range of 4–15°C (Table 3). As was expected, significantly paler colour was observed (p < 0.05) at the highest temperature, as compared to the low and intermediate temperatures. Additionally, significantly lower lightness was obtained for stretched muscle as compared to the unrestrained control, when the

TABLE 3				
Lightness (L*) of Porcine Biceps Femoris Muscle Strips as Influenced by Pre-rigor M	uscle			
Stretch and Rigor Temperature				

Temperature (°C)	Stretched	Unrestrained
4	(11) 47.7 <sup>a</sup>	(14) 48.3 <sup>a</sup>
15	(9) 47.4 <sup>a</sup>	(8) 49.3 <sup>a</sup>
36	(9) 55.6 <sup>a</sup>	$(12) 60.0^{b}$

<sup>*a,b*</sup> Means of the same superscript are not significantly differ within the same rows (p < 0.05) (Student's *t*-test). In () numbers of replicate measurements are given.

TABLE	4
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Least-square Analysis of Salt Extractable Myofibrillar Protein and Swelling of Myofibrils Prepared from Porcine *Biceps Femoris* Muscle Strips Either Stretched or Left Unrestrained and Subjected to Different Rigor Temperatures

Source of variance	DF	Extractable protein	Swelling
Pre-rigor stretch (P)	1	NS	NS
Temperature (T)	2	<i>p</i> < 0.001	p < 0.001
Duration of extraction/swelling(D)	1	p < 0.001	<i>p</i> < 0.001
$P \times T$	2	p < 0.001	p < 0.001
$P \times D$	1	NS	NS
$\mathbf{T} \times \mathbf{D}$	2	<i>p</i> < 0.001	NS
$P \times T \times D$	2	NS	p < 0.05
RSD	12	1.26	0.74

RSD = residual standard deviation.



Fig. 6. Effect of stretch and duration of extraction on myofibrillar protein extraction after muscle incubation at different temperatures: (a) stretched (●), unstreched (○); (b) extraction duration (△) 15 min, (▲) 1 day. In (a) each point represents the mean of two levels of extraction period each of two replicates. In (b) each point represents the mean of two contraction levels each of two replicates.

\* Means significantly differ between stretched and unrestrained samples (a) or 15-min and 1-day extractions; (b) (p < 0.05).

highest temperature prevailed during the development of rigor mortis (p < 0.05). This phenomenon accorded with the initial retardation of pH fall in stretched muscle (Fig. 1). The latter can be assumed to cause less severe sarcoplasmic precipitation (not determined in the present study).

#### Extraction and swelling of myofibrils

The main effects of temperature, and of the time of extraction, were significant, when extractable protein and swelling were determined in the presence of 1.0 M NaCl (p < 0.001) (Table 4). The interaction of stretch × temperature was also significant (p < 0.001) in respect of both extractable protein and swelling. Furthermore, the interaction of temperature × time of incubation was significant for extractable protein (0.001), while stretch × temperature × time of incubation was found to be significant in the case of swelling only (p < 0.05).

The yields of extractable protein, for stretched and control myofibrils, are presented in Fig. 6a, as affected by temperature. Although the solubility of myofibrillar protein was generally strongly reduced by high temperature treatment, it was significantly better preserved during storage at 36°C (p < 0.05), when the muscle was unrestrained and allowed to shorten. In contrast, an inverse tendency was observed at 15 and 4°C: it was the stretched muscle that showed significantly (p < 0.05) better solubility.



Fig. 7. Swelling of myofibrillar protein as influenced by pre-rigor stretch and rigor temperature at two extraction periods ( $\bigcirc$  = stretched,  $\bigcirc$  = unrestrained). Each point represents the mean of two replicates.

\* Means significantly differ between stretched and unrestrained samples (p < 0.05).

The yield of extractable protein for all muscle samples was strongly enhanced by the prolonged extraction particularly at 4 and 15°C (p < 0.001) (Fig. 6b).

It is obvious from Fig. 7 that swelling followed the same pattern as the solubility did in relation to stretch and temperature. Thus, there was maximum swelling of myofibrils prepared from stretched strips held at 4°C, while minimum swelling occurred in the case of stretched strips exposed to 36°C. In contrast to extractability, swelling approached its maximum value rapidly (15 min) even in the case of low and intermediate temperature treatment and showed only a moderate but still significant (p < 0.001) increase during a subsequent incubation of 1 day. Figure 8 illustrates the positive linear relationship between swelling and extractability of myofibrils calculated by using the combined data of stretched and unrestrained samples: r=0.85 (p < 0.05) and r=0.95 (p < 0.01) were obtained



Fig. 8. Relationship between extractable myofibrillar protein and swelling of myofibrils for different extraction periods ( $\bigcirc = 1$  day unrestrained,  $\bigcirc = 1$  day stretched,  $\triangle = 15$  min unrestrained,  $\triangle = 15$  min stretched). Each point represents the mean of two replicates.

for the 15-min and 1-day incubation periods, respectively. These results accord those of Penny (1969), who reported a strong correlation between myofibrillar extractability and swelling.

#### DISCUSSION

Retardation of the post-mortem decomposition of energy stores in overstretched muscle (Figs 1 and 2) can be explained by the reduced ATP-ase activity of actomyosin, due to the limited overlapped area of thick and thin filaments when formation of actomyosin is physically inhibited. The present results are in accordance with that of Ward *et al.* (1965) who found greatly reduced ATP hydrolysis in frog muscle at large sarcomere lengths (2.2-4.0 mm).

The relationship between ATP-depletion (*R*-value) and pH fall were reported to be linear in the study of bovine neck muscle (Honikel *et al.*, 1983) and in ovine back muscle (Beltrán *et al.*, 1993). These authors observed markedly slower pH fall at 0°C and -1 to -2°C, respectively, as compared to temperatures in the range 4-36°C. In the present study with porcine leg muscle, when the pH was plotted against the *R*-value, appreciable slowing of glycolysis was found at 4°C as compared to the rate of ATP breakdown (Fig. 3). This finding suggests that both pH and *R*-value should be measured when studying the rate of post-mortem metabolism (at least in the low temperature range).

The results obtained for bovine (Locker & Hagyard, 1963) and ovine muscle (Beltrán *et al.*, 1993) suggested that the extent of cold shortening was greater than that of rigor shortening at body temperature. Hertzman *et al.* (1993) found a greater extent of hot rigor shortening in the bovine muscle, which is more oxidative. The porcine *biceps femoris* studied here showed nearly identical cold and hot rigor shortening (Fig. 4), which accords with the results which Honikel (1986) obtained for pork. Further data will be needed to elucidate the temperature-dependence of muscle shortening with respect to the fibre types present and their accompanying metabolism.

The relative superiority of unrestrained muscle exposed to high temperature, in terms of myofibrillar extractable protein and swelling (Figs 6 and 7), can be explained by the formation of a large number of rigor bonds caused by the more complete overlap of myofilaments before and during shortening. In contrast, in the stretched muscle, a limited number of rigor bonds are formed, consequently the major part of myosin would denature during storage at high temperature despite the lower rate of pH decline (Fig. 1) and the longer survival of ATP (Fig. 2). The strong protective effect of actin against denaturation of myosin was suggested by Penny (1967) and Offer (1991), while ATP is known to possess a limited stabilizing effect (Blum, 1960). On the basis of kinetic calculations, Offer (1991) instanced pre-rigor acidification at high temperature as conducive to poor meat quality. The results presented here provide further evidence for the stabilizing effect of rigor bonds and point to the enhanced sensitivity of stretched muscle to acidification at high temperature. In the case of inadequate chilling, denaturation of myosin would continue in post rigor stretched muscle. The high drip loss of pelvic suspended and electrically stimulated m. semimembranosus, observed by Taylor et al. (1992) can also be explained by enhanced sensitivity of myosin to acidification.

Extreme conditions, including stretch accompanied by rapid pH decline, may have practical impact for certain muscles of the pelvic limb of PSS pigs in which acidification probably starts *ante mortem* (Essén-Gustavsson *et al.*, 1988; Estrade *et al.*, 1991) and proceeds rapidly during bleeding and scalding when these muscles are still stretched, due to the natural position of pelvic limb. Also, *m. psoas major* is known to be strongly stretched in the Achilles-suspended carcass. The elevated muscle temperature of PSS pigs will strongly contribute to rapid denaturation of myosin even before the ultimate pH is attained.

The relationship between the solubility properties of contractile protein and tenderness in relation to sarcomere length was recognised in the early work of Partman (1963) and Hegarty *et al.* (1963). The high percentage of soluble protein in pre-rigor stretched beef was found to be unchanged during post-mortem storage over 2 days at 7°C (Cook, 1967). Similarly, there was a lower yield of extractable protein (in the presence of 0.6 M KCl) obtained with contracted, in comparison with uncontracted myofibrils (Nagy-Németh & Vada-Kovács, 1979). The higher drip loss of cold shortened pork (Honikel & Reagan, 1987; Dransfield *et al.*, 1991; Taylor *et al.*, 1992), as well as the less acceptable processing properties of rapidly chilled, in comparison with pelvic suspended ham (Smulders *et al.*, 1992; Vada-Kovács, 1993) are in accordance with the present results, suggesting that there is a better hydration capacity in stretched muscle when adequate chilling is applied. It has been also established in the present work, that the colour of chilled pork seems to be unaffected by muscular stretch, although hydration capacity was significantly improved.

According to the hypothesis for the mechanism of swelling and solubilisation when based on microscopic observation of unshortened myofibres (Offer & Knight, 1988), solubilisation is preceded by swelling of the A-band. In the course of the swelling process thick filaments were assumed to be loosened, followed by the attachment of myosin molecules, located in the overlapped region, to the thin filaments by their free end. In contrast, myosin molecules in the region of H-zone were believed to solubilize quickly. It can be concluded, from this hypothesis, that swelling and extraction would be inversely related in the particular case of overstretched myofibrils having a large region of H-zone, because low swelling would be accompanied by high solubility. The present result obtained, with samples kept at low pre-rigor temperature, does not appear to confirm this idea, because stretched myofibrils showed higher swelling, together with higher solubility after a short incubation with salt solution in comparison with the markedly shortened control myofibrils (Fig. 7 and Fig. 8). Pre-rigor stretched muscle bundles can serve as adequate model for studying structural changes during swelling and extraction of myofibrils of large region of H-zone.

It should be noticed, that proteolytic modification of the myofibrillar structure would not be expected to result in better solubility of shortened myofibrils at high temperature, because a low ultimate pH was attained 1.5 h earlier in shortened muscle. This would allow less time for the action of neutral proteases which, otherwise could lead to better hydration.

The results obtained in the present study demonstrate that paleness does not appear a reliable predictor of hydration properties, at least for PSE of differing severity, because the  $L^*$  value, and the yield of extractable protein, are definitely influenced by muscle length (Fig. 9.). Since the precipitation of sarcoplasmic protein is undoubtedly associated with paleness (Lundström *et al.*, 1988; Lopez-Bote



Fig. 9. Relationship between yield of soluble myofibrillar protein extracted for 1 day and the lightness of muscle ( $\blacksquare$  = stretched,  $\blacktriangle$  = unrestrained).

et al., 1989; Von Seth et al., 1991), the higher  $L^*$  value obtained for shortened muscle which had been kept at the higher temperature (Table 3) can be attributed to the more rapid pH decline (Fig. 1), and consequently to a higher degree of sarcoplasmic precipitation (Sayre & Briskey, 1963; Scopes, 1964). Unfortunately, the relative contribution of sarcoplasmic precipitation and other structural factors in muscle to paleness has not yet been elucidated (Swatland, 1993).

Although paleness generally indicates low water holding capacity, the opposite tendency has been found in the particular high temperature and low pH resulting in a combination of pale colour and better water holding capacity. Similarly, moderately low  $pH_1$  (higher than minimum ultimate pH) can be associated with extreme low hydration, while low  $pH_1$  and very pale colour can be accompanied by moderately reduced hydration.

Besides elucidation of the varying correlations of the above mentioned quality attributes, the present results may contribute to a better understanding of contradictory data concerning the sarcomere length in PSE (Larsson & Tornberg, 1988), since the present results suggest that varying sarcomere lengths in PSE pork seems to be a real phenomenon: the severity of PSE character might be associated with differences of muscle length.

Further investigation are planned on the relation between drip loss and muscle length at higher temperature.

With respect to physical inhibition of complete development of rigor shortening, it seems to be desirable to investigate the sarcomere length of pork of intermediate qualities like PFN, RSE. This was unfortunately omitted in the extended study of Kauffman *et al.* (1993). A more objective definition of softness/ firmness and 'floppiness' is also needed.

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