CHAPTER 9

PRE-RIGOR CONDITIONS IN BEEF UNDER VARYING TEMPERATURE- AND PH-FALLS, STUDIED WITH RIGOMETER, NMR AND NIR

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ABSTRACT

Beef M.longissimus dorsi (LD) were subjected to three different glycolytic rates during rigor (fast: pH 5.6, 4-5h post-mortem; medium: pH 5.6, 12h p.-m.; slow: pH 5.6, 20 h p.-m.) combined with two chilling regimes (20°C, 5h p.-m.; 12°C, 5h p.-m.) each in a factorial experimental design. Spectroscopic techniques, such as Nuclear Magnetic Resonance (NMR) and Near InfraRed (NIR), were used together with pH-, shortening (SH) and isometric tension (IT) measurements to characterise the meat during the development of rigor.

Meat with the fastest pH drop created larger extra-cellular volumes and cell membranes were destroyed, giving rise to a leakage of sarcoplasmic proteins at an earlier stage of the rigor process. This behaviour occurred later in the rigor process in meat subjected to the slower pH drop. These evaluations were based mainly on NMR measurements, while NIR spectroscopy did not contain any significant information about this issue.

The fast pH group gave the most tender meat at fully developed rigor and measured as W-B shear values, although shortening was here among the highest. The reason for the superior tenderness of the fast pH group is suggested to be caused by a quicker and a more substantial proteolytic break-down for this type of meat compared to the more slowly glycolysing muscles.

INTRODUCTION

Tenderness is considered by the consumer to be one of the most important quality criteria for beef. Consequently, this property of meat has been the subject of considerable research over the years with the aim to be able to measure, predict and control it, especially under processing conditions. The research was in the past more directed towards the understanding of different mechanisms governing the development of tenderness, whereas in recent years the predictability of tenderness early post-mortem has been a subject of special interest.

Early post-mortem development of rigor takes place and to study and understand this process is of vital importance when trying to predict tenderness of meat in the early stages of the slaughtering process. During rigor shortening of muscle fibre occurs and is detrimental to tenderness. The degree of shortening is dependent not only on chilling conditions but also on the stress ante-mortem and the course of pH prior to fully developed rigor.

Locker and Hagyard (1963) found, when studying rigor development of M. sternomandibularis at different constant temperatures that there exists both cold- and warm-shortening below and above 15 C and 20 C, respectively. This work has been extended to cover more commercially relevant muscles, such as M. longissimus dorsi (LD) and M. semimembranosus (SM), using a rigometer able to record both shortening (S) and isometric tension (IT) during the development of rigor (Hertzman et al., 1993; Olsson et al., 1994; Devine et al., 1999). They showed a temperature region of minimal shortening at 10-15 C for the LD muscle and 7-13 C for the SM muscle.

From a study of S and IT during rigor it was suggested by Wahlgren et al., 1997 that both the muscle shortening and the proteolytic degradation occuring during the rigor process could be recorded. Taylor et al.,(1995) have shown that the greatest changes in proteolytic degradation caused by the proteases, named calpains, occur within the first 3 or 4 days post-mortem, i.e. including the rigor period. The extent of degradation is critically dependent upon the net proteolysis resulting from the activity and the inactivation of calpains (Dransfield, 1994). The inhibitor calpastatin may also be important in controlling calpain activty and its proteolysis (Wheeler et al., 1990; Koohmaraie et al., 1991). Simmons et al., 1996 have studied the activity of calpains at different constant temperatures above and equal to 15 C and found that the activity decreases during rigor but least at 15 C.

The studies on constant temperatures during the development of rigor have been followed by a study of the influence of different temperature-time courses on S, IT and tenderness in beef LD (Wahlgren et al., 1997). The different chilling regimes studied gave rise to varying appearance of the shortening-time-dependence, but the degree of shortening was reduced as compared to the results obtained at constant temperatures in the cold- and

warm-shortening regime. However, the sensory tenderness after 7 days' ageing differed among the various chilling regimes, with those at 12 C and 20 C after 5 hours being the best. The variation in tenderness observed was suggested to originate from a combination of different degrees of shortening and proteolysis.

Ultimately, different rates in glycolysis (causing varying pH courses) have an impact on the resulting tenderness, although the mode of influence varies from investigation to investigation. Glycolytic rate can vary widely from animal to animal (Kauffman and Marsh, 1987; O'Halloran et al., 1997). One way to control the rate of glycolysis is to electrically stimulate (ES) at various times after slaughter. Chrystall et al., (1980) concluded that ES should be applied as soon as possible after slaughter to elicit nervous responses, thereby creating the quickest pH falls. The problem with most of the investigations in this area is that there exists a confounding effect of the concomitant temperature decline. Not only does the temperature fall in itself influence the pH decline but also an early attainment of low pH at elevated temperatures (> 15 C) can cause denaturation and/or autolysis of the enzymes and therefore a decreased tenderness. This was suggested to be the cause of the results presented by Wahlgren et al., 1997, where the medium pH-time course (pH 5.6 , 10 h p.m.) gave the best tenderness 3 days p.m. in comparison with the quickest pH fall (pH 5.6, 1h p.m.). This phenomenon seems to be a plausible explanation to an optimum in pH decline often observed during rigor in relation to the ultimate tenderness of the meat, especially at the early stages of the ageing process (Geesink et al.,1994; Takahashi et al., 1984; Shackelford et al.,1994; Marsh et al., 1987). The only investigation so far to evaluate the influence of different glycolytic rates on the tenderness of LD muscle without the confounding effects of varying temperature falls was performed by O'Halloran et al., 1997. They found that the fastest glycolysing muscle (pH 5.6, 6h p.m) gave the most tender meat and they suggested it to be caused mainly by increased proteolysis.

In the present study both the temperature- and pH-fall during rigor were varied and controlled according to a factorial experimental design, i.e. the experiments were performed at two realistic temperature-decline (12 C and 20 C at 5 h), with three different pH falls each (pH 5.6 4-5 h p.m.; pH 5.6 12 h p.m.; pH 5.6 20 h p.m.) The variation in pH decline was obtained by different types of ES, whereas in the study by O'Halloran et al., (1997), the muscles were selected into different pH-groups according to their natural variation in glycolytic rates. Moreover, in the present study a number of methods have been used to characterize the rigor process more thoroughly. Besides the Rigotech measurements (S and IT), pulsed ^{1}H low-field Nuclear Magnetic Resonance (NMR) and Near InfraRed (NIR) measurements were performed during rigor development. At fully developed rigor (24 h) also Myofibrillar length (MFL) and Warner-Bratzler shear force measurements (W-B) were added to the registrations.

A powerful tool for studying the distribution of water in muscle is the use of the non-invasive NMR. Water protons in meat have shorter transverse relaxation times (T_2) than in bulk water. Zimmerman and Brittin, 1957 have given an explanation to this behaviour, suggesting that there exists a fast exchange between free water and the hydration water adjacent to proteins. A long relaxation time suggests a long diffusion distance of the free water protons to the exchange site. This means that water in larger pores within the muscle structure have relaxation times in the proximity to that of free water, whereas water in smaller pores will have much shorter relaxation times. Therefore, the pore-size distribution of water in the muscle can be studied. However, it has been observed (Halle et al., 1981; Belton and Wright, 1986) that the relaxation time of the hydration water is only 3-10 times shorter than that of free water, which cannot account for the dramatic increase observed in relaxation rate, when proteins are present in the solution. An explanation suggested by Hills et al., 1990, is the cross-relaxation involving non-water protons in the protein molecules. These observations do not necessarily rule out the above mentioned elucidation of the NMR measurements. Whilst they do not give useful information about the state of water they do give valuable information about the morphology of the protein system(Hills et.al., 1989),.

The multi-exponential decay of the T_2 of water protons in muscle, especially pork muscle, has been reported in the literature (Renou et al., 1985; Fjelkner-Modig and Tornberg, 1986; Larsson and Tornberg, 1988; Renou et al., 1989; Borisova and Oreshkin, 1992). Two dominating, discernible relaxation processes are generally observed, where the major fraction (80%) of the muscle water has a T_2 between 35-50 ms, whereas the remaining water relaxes in the range of 100-150 ms. The fraction of water relaxing with the shortest relaxation time can be considered as mainly intracellular water, since a very high fraction of the water is situated within the myofibrils. These discrete water domains do not necessarily have to arise from the structural domains seen in histological pictures of meat (Lillford et al. 1980). However, when comparing the percentage of water with a T_2 = 100-150 ms with the percentage of water around the fibre bundles (evaluated by microscopy), Tornberg and Larsson, 1986 found a high correlation.

NIR has been mainly for the quantitative analysis of foods (Osburne and Fearne, 1986), but also the ability of NIR to predict quality parameters in foods, such as the tenderness of beef, has also been explored (Hildrum et al., 1994; Park et al., 1998). But the predictability of NIR measurements for tenderness of meat has not yet been carried out at the early stages of the post-mortem period, i.e. during the rigor process. In this paper results from such measurements will be presented.

Due to the experimental design, the variety of analytical techniques applied and the vast spectral resolution, large amounts of data have been collected in this study. Chemometrics is an efficient way of analysing such complex and multivariate data. In contrast to traditional statistics, chemometrics is capable of dealing with the colinearity in especially

the spectral data. The most important techniques in chemometrics have been explained for use in meat quality by Næs *et al.* (1996).

The aim of present work was to study the post mortem (p.-m.) changes in meat in a full factor design experiment involving three pH falls and two temperature profiles at 5 different post mortem times (1.5 h, 3 h, 5 h, 7 h, and 24 h). In each point of the factorial design the changes in the meat was measured by physical measurements (isometric tension, myofibrillar length, sarcomere length, Warner-Bratzler, and cooking loss), spectroscopic measurements (NIR and NMR) and chemical measurements (pH). The data is evaluated chemometrically to study the glycolytic development in the samples and the relations between the measured techniques.

MATERIALS AND METHODS

Meat samples of *M. longissimus dorsi* (LD) were taken from 22 young bulls of the Swedish Lowland breed. To achieve three different pH courses during rigor development, *fast, medium* and *slow,* the carcasses were subjected to different types of electrical stimulation:

(1) no stimulation

(2) low voltage electrical stimulation applied 15 min post-mortem (80V, 15 Hz, 5 ms, 48 s)

(3) low voltage electrical stimulation applied 30 min post-mortem (80V, 15 Hz, 5 ms, 30 s)

Both of the LD muscles from each animal were excised 45 min. post mortem and received at the research facility within 1 hour post mortem. The whole muscles were cut into approximately four 10-15 cm pieces, which were placed in plastic bags and vacuum-packed, then immersed in a water bath and chilled according to two different chilling regimes (12 C and 20 C at 5 h p.m., respectively).

The progression into rigor was monitored by direct pH measurements (1.5, 3, 4, 5, 6, 7, 8, 12, 24 hours post-mortem) using a combination puncture electrode (Mettler Toledo) on muscle samples not vacuum-packed.

To follow the muscle shortening and the increase in muscle tension continuously during rigor development strips of muscle, longitudinally oriented along the fibre axis, approximately 35 mm long and weighing 1.5-2 g, were cut out of the LD using trimming blades for pathological operations (Feather Safety Razor Co. LTD. Japan). The strips of muscle were attached to the isometric and isotonic recording options of the rigormeter (Rigotech**,** Reologica Instruments AB, Sweden) using cyanoacrylate glue (Loctite®, superglue) and were covered with paraffin to avoid drying out and to exclude oxygen (Hertzman *et al*., 1993). The isometric tension, IT, expressed as force per unit area, and muscle shortening, SH, expressed as percentage decrease in the length of muscle piece,

were registered every 5 min. All measurements were carried out in triplicate in a closed chamber at a controlled temperature decline to an accuracy of ± 0.5°C.

Small pieces of muscle (approximately 1-2 grams) were cut out at selected time intervals (1.5, 3, 5, 8 and 24 hours *post-mortem*) and subjected to NMR-measurements, which were performed by measuring the transverse relaxation time (T_2) of the water protons using a Maran, bench-top pulsed NMR analyser (Resonance Instruments, Witney United Kingdom) operating at a frequency of 20 MHz. T_2 was measured using a CPMG-sequence with a τ-spacing of 150 μs. The signal amplitude (M) was measured every second echo, in total 1024 data points. 16 acquisitions were accumulated with a repetition time of 4 s. The CPMG relaxation curves were fitted both to a one-component exponential model [1] and a two-component exponential model [2]. A three component model was judged as an overfitting of the recorded data.

$$M = M_0 * e^{-t/T_2} \qquad [1]$$

$$M = M_0 (p_{21} * e^{-t/T_{21}} + p_{22} * e^{-t/T_{22}}) \qquad [2]$$

where M_0 and M are the magnetisation amplitude at time, t=0 and t=t, respectively. p_{21} and p_{22} are the fractions of water within the meat in different environments and T_{21} and T_{22} are the corresponding characteristic transverse relaxation times.

NIR measurements were made on meat slices of 1.5 cm thickness and a 4.5 cm diameter with a NIR Systems Inc. (model 6500, Silver Springs, Maryland, USA), covering the wavelengths from 400 to 2498 nm (thus also including the visual region). At every 2nd nm a registration was performed giving a spectrum of 1050 points. The spectrophotometer uses a split detector system with a Silicon (Si) detector between 400-1100 nm and a Lead Sulfide (PbS) detector from 1100 to 2498 nm. The angle of the illuminating light source was 180° and reflectance from the meat surface was measured at a 45° angle. The NIR reflection spectra were recorded using a rotating sample cup with a quartz window, and spectral data were converted to log(1/R) units. The spectra was subsequently scatter corrected using Multiplicative Scatter Correction (MSC) prior to PCA analysis (see Martens and Næs, 1993).

After fully developed rigor (24 hours post-mortem) samples were cut into 1.5 cm thick slices and fried to an internal temperature of 70°C. Loss of weight on cooking loss was determined as the percentage weight loss on frying by weighing the slices of meat before and after frying.

The meat was further cut into pieces for measurements of Warner-Bratzler (W-B) shear force (3cm) and myofibrillar length (MFL) (10g).

The Warner-Bratzler *shear force* measurements were undertaken using an Instron® Universal testing machine (4301) equipped with a modified Warner-Bratzler shear blade with a square opening of 26x21 mm and a blade thickness of 1.0 mm. The meat was

cooked in a water bath at 74°C for 60 min and thereafter chilled to room temperature in ice. The maximum shear force for at least 10 pieces (0.7x1.5 cm), sheared across the direction of the fibre, was recorded.

Myofibrillar lengths were determined using a method developed at the Swedish Meat Research Institute (Olsson and Tornberg, 1992). A small sample of *Longissimus* muscle (about 5 g) was cut into small pieces using a knife. 50 ml of an isolation medium (I-medium: 100 mM KCl, 20 mM K phosphate, 1 mM EDTA, 1 mM NaN_3) was added to the minced sample. The preparation was homogenised in an omnimixer for 60 s at 11.000 rpm. The homogenate was centrifuged at 2°C for 15 min at 1.000 g. After discharge of the supernatant, 25 ml of I-medium were added to the sediment, and the samples were brought into suspension. One ml of the suspension was further diluted with 25 ml of I-medium. One drop of this suspension was mounted onto a microscope slide. A light microscope (Nikon Optiphot), using phase contrast with standardised magnification (x1340), equipped with a camera, "Sony 3 CCD", was used. The pictures taken were analysed with an image analysis system (Image Pro Plus 3.0, Media Cybernetic, USA) to determine the length of the myofibrillar fragments.

Sarcomere lengths were measured directly by using light microscopy (Nikon Optipot), video images (Sony3 CCD) and an image analysing programme, the Image Pro Plus 3.0 (Media Cybernetic, USA) on single fibers prepared by fixing in 2 % glutaraldehyde and carefully teared out.

Samples of the meat, with the outer connective tissue sheath removed, were taken for chemical analysis of fat-, protein-, moisture- and connective tissue content (Hertzman et al., 1993).

The investigation was designed as a 2*3 factorial experiment with three replicates. The variables, chilling regimes and pH courses, were studied at two and three levels, respectively. When appropriate, ANOVA and tukey's test were performed using the SYSTAT 5.04 program (SYSTAT, Inc., Evanston, IL, USA).

Variations in and relations between spectral and chemical data are analysed using Principal Component Analysis (PCA) (Wold, Esbensen, and Geladi, 1987). PCA utilises a two-dimentional data evaluation strategy in which the information is extracted from a matrix of multivariate instrumental data defined by the meat sample (factorial design point). The essence of PCA lies in the construction of orthogonal latent factors (or principal components) from underlying latent structures in the original data. In PCA the two-dimensional data matrix is decomposed into systematic variation and noise. The systematic variation is described by the principal components (PC1, PC2 etc.) which each represent the outer product of scores and loadings. The scores are related to the samples whereas the loadings are related to the variables (e.g. wavelengths). After PCA decomposition, the samples are separated only by a multiplicative factor called scores and the score values can be seen as a new much simpler set of variables for representing the

samples. Thus the scores can be used to detect eventual grouping tendencies in the data whereas the loading vectors can be used to detect interactions between the original variables. All multivariate data studies were performed in Unsrambler 6.2 (CAMO, Trondheim, Norway) and Matlab 5.2 (Mathworks Inc., Natick, MA, USA).

In this dataset first a PCA was performed on the NIR spectra and the complete NMR relaxation data separately. To simplify the interpretation of the further data analysis with the entire variable set, the score values from the initial PCA were used instead of the entire spectra. Thus, a second PCA including loadings from the NMR relaxation curves and the NIR spectra together with the IT-, SH- and ph-data was performed at different times post-mortem.

RESULTS AND DISCUSSION

pH- AND TEMPERATURE-FALL

The two chilling regimes are linear up to 5 hours post-mortem, when the slower reached 20 C and the quicker 12 C (Figure 1a). From 5 to 12 h the linear time-temperature slope diminished, reaching the same temperature of about 9 C at 12h for both chilling regimes.

The different types of electrical stimulation gave essentially three different rates of pH fall during rigor development (Figure 1b). The three pH-time courses, *fast* (reaching pH 5.6 after 4-5 hours post-mortem), *medium* (reaching pH 5.6 after 12 hours post-mortem) and *slow* (reaching pH 5.6 after 20 hours post-mortem) differed significantly from each other ($p<0.001$). Although care was taken to minimise animal stress, one animal produced meat with a high ultimate pH ($pH_u=6.12$) and was therefore deleted in the further analysis. The slower chilling regime gave a somewhat faster pH fall for all three groups (Figure 1b). However, the differences were not significant.

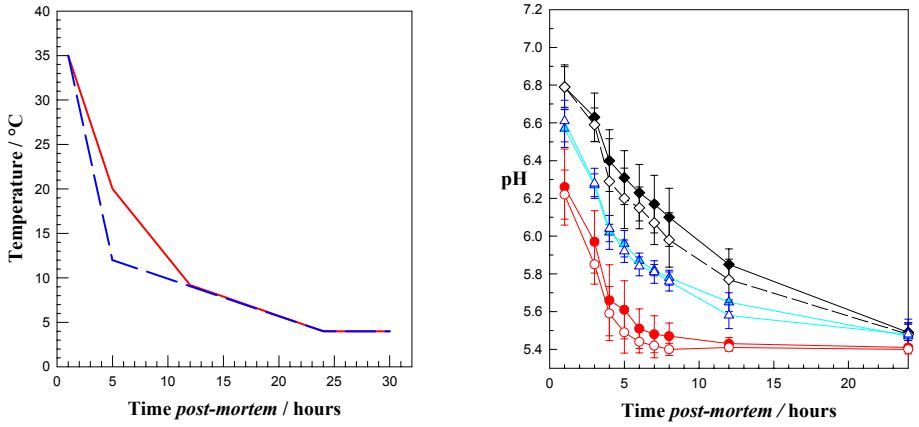

Figure 1.Left) The two chilling regimes used in the experiment. Right:) The pH-fall as a function of time post-mortem for the fast (●, ○) medium (▲,△) and slow (◆,◇) pH-courses. Filled symbols are fast chilling (20°C, 5h) and open symbols slow chilling (12°C, 5h).

MUSCLE SHORTENING AND ISOMETRIC TENSION

The development of rigor at the two temperature- and the three pH-falls can be studied in Figure 2 a-d. A large animal to animal variation was sometimes observed. A significant difference, however, was observed in shortening at the slowest temperature fall (20°C, 5 h) for varying pH courses (using ANOVA). The medium pH drop gave rise to least shortening compared to the slow and the fast drops. The highest isometric tension (IT) was also achieved with the fastest pH fall. For the slowest temperature fall (12°C, 5 h) the same order in maximum IT achieved was observed for the three pH courses as at 20°C, 5h, i.e. the fastest pH drop had the highest maximum IT and the slowest pH fall had the lowest IT. Also for muscle shortening, the same ranking order for the three pH courses can be observed at the two different temperature falls. However, in this case the medium pH drop gave the lowest degree of shortening and the slow and fast pH drops gave rise to a higher and nearly equal amount of shortening. It can also be stated that generally the degree of shortening and IT was larger at the higher temperatures. This observation can probably be ascribed to the phenomenon of warm-shortening (Hertzman et. al.,1993). We have observed, in another investigation (Olsson *et al.*, 1995) that the higher the ultimate pH the larger the shortening. In this study the pH course during rigor is different but the ultimate pH is not. The reason for the lowest shortening at the medium pH course is unexpected and difficult to explain. The ranking order in IT with regard to the pH courses is perhaps easier to grasp. The development of rigor is quicker in the fast pH drop group and therefore there is a higher probability for more fibres to go into rigor at a higher temperature and to achieve a larger degree of warm-shortening.

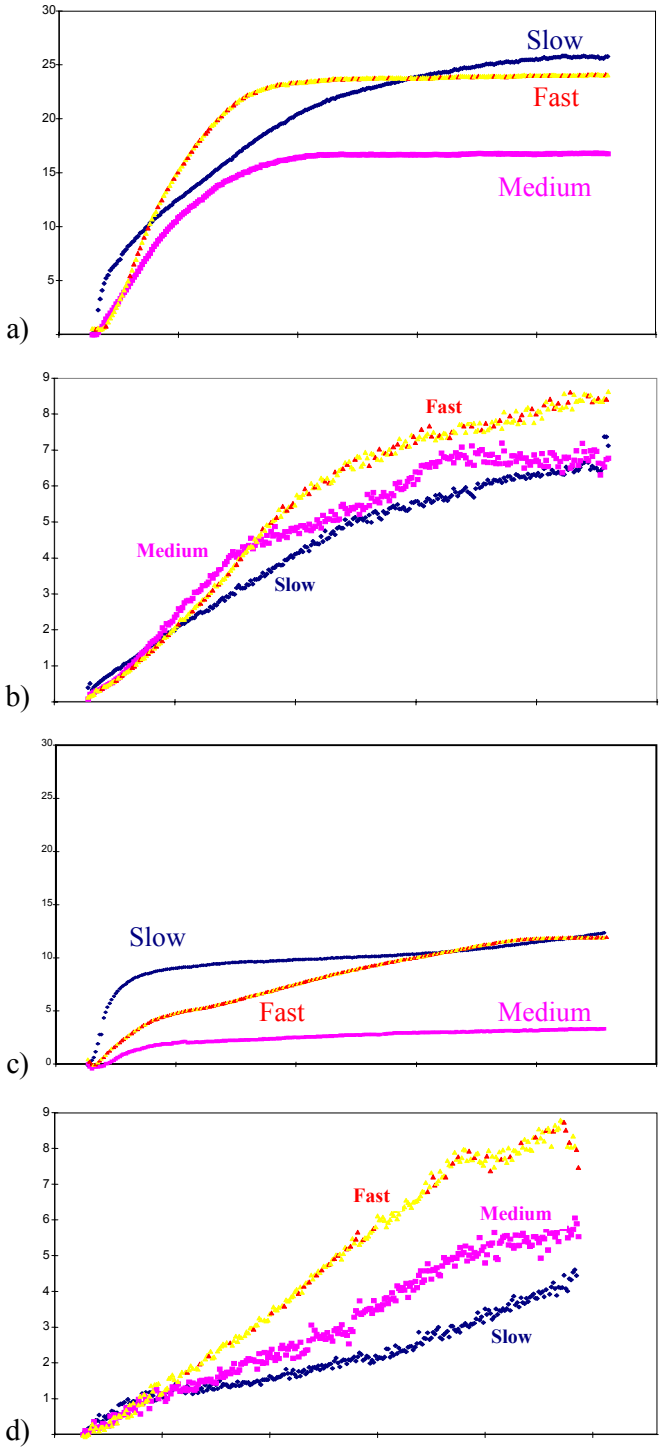

Figure 2. The course of shortening SH (%) (a, c) and isometric tension IT (kpa) (b, d) during rigor development at a temperature fall of 20 (a, b) and 12°C (c, d) after 5 hours. *Slow, medium*, and *fast* represent the three different pH drops studied, i.e. *slow*: pH 5.6 after 20h; *medium*: pH 5.6 after 12h; *fast*: pH 5.6 after 4-5h.

NMR

The NMR measurements showed that during early post mortem the CPMG relaxation curve could be described by a one-component system. However, during the development of rigor there was an increased tendency that a two-component system described the CPMG relaxation curve better. We therefore used a two-component system in all the NMR evaluations. The fast component had a relaxation time (T_{21}) varying from 40 to 52 ms Figure 3a), which is in accordance with earlier observations. This was also the case for the slower component (T_{22}), which had a variation of about 100-300 ms (Figure 3c). The number of protons, p_{21}, relaxing according to the quickest relaxation time is somewhat higher compared to results given in literature, in which only post-rigor has been analysed (Figure 3b).

During development of rigor there was first an increase in the relaxation time, T_{21}, of the fast component, followed by a decrease during the ageing period for the *slow* and *medium* pH groups (Figure 3a). The group with a *fast* rigor development, however, started with a relatively high relaxation time, which decreased during the whole period. For the two slowest pH falls, the number of protons, p_{21}, with the quick relaxation time of about 40-50 ms, were practically unchanged until the maximum in T_{21} was reached, after that a decrease was observed (Figure 3b). In the case of the fast pH drop, however, a continuous drop in p_{21} over the whole period was noted. A significant difference in this post-slaughter decrease in p_{21} among the three groups was not seen. It is also interesting to note that T_{22} during the first 24 h p.m. is in general larger for the two slowest pH fall courses compared to the fast pH drop.

If the fraction with a fast relaxation rate is assumed to originate from water protons within the muscle fibres and the fraction of slow relaxation rate originates from external water, then the results suggest that there is a transport of fluid from the inside of the muscle fibres out to the spaces between the fibres during rigor development. Tornberg *et al.* (1993) have shown that these assumptions may be valid, because a linear relation was observed between T_{22} and the square distance between fibre bundles in raw porcine LD muscle of different qualities. This dependence shows that the migration of the protons to the exchange site is diffusion-controlled and that this diffusion distance is similar to the extra-cellular distance between fibre bundles.

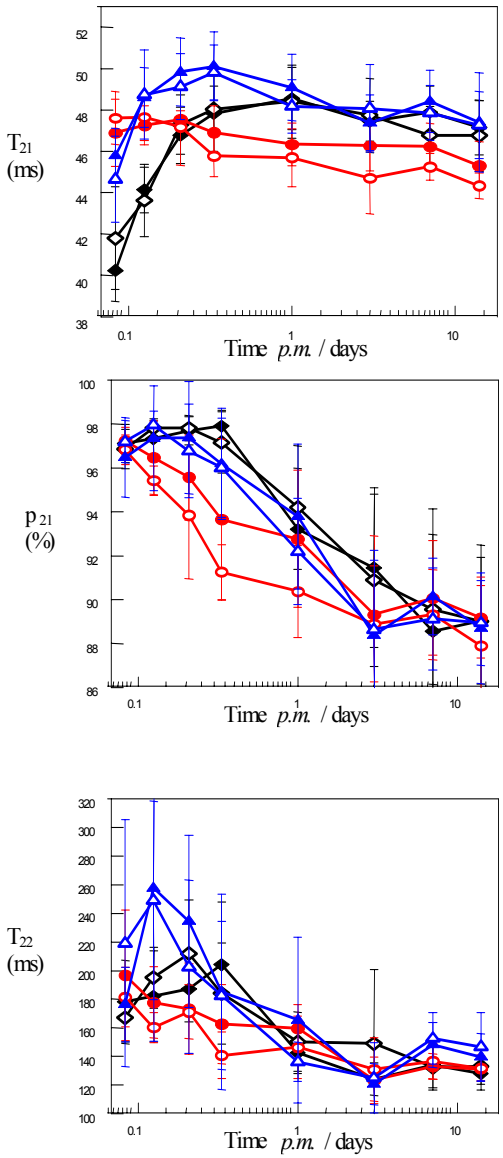

Figure 3. ***a)****T_{21} as a function of time post-mortem, pH-time and temperature-time regime during rigor and ageing. Legends as in Figure 1.* ***b)****p_{21} as a function of time post-mortem, pH-time and temperature-time regimes during rigor and ageing. Legends as in Figure 1.* ***c)*** *T_{22} as a function of time post-mortem, pH-time and temperature-time regimes during rigor and ageing. Legends as in Figure 1.*

With this reasoning in mind the time-courses of the NMR-data shown in Figure 3 can be further evaluated. T_{21} can accordingly be assumed to be the relaxation time for the protons within the fibre and, the more dense the protein matrix or the higher the concentration of proteins are within the fibre the lower the T_{21}. A lower T_{21} can therefore also suggest that the cell membranes are more intact and that therefore fewer sarcoplasmic proteins have leaked out in the extra-cellular space. We know that during rigor the cell membranes are

partly destroyed and the quicker the rigor is developed probably the faster the leakage of the sarcoplasmic proteins occurs. This is probably the case for the fast pH drop. A higher intra-cellular protein concentration of the two slower pH-groups could then be one of the reasons for the lower T_{21} compared to the fast pH drop at the very early stages of the post-mortem process.

T_{21} decreases with prolonged time post-mortem in the case of the fast pH fall, whereas for the other two pH groups first a swelling of the myofibrillar space takes place up to about 8 h p.-m. This swelling is probably due to the continuous contraction taking place, which can be observed in Figure 2. That the cell membranes are still intact up to 8 h p.-m. and therefore no leakage of the sarcoplasmic proteins has occured is suggested by the high content of protons in the myofibrillar space (p_{21}) and the slow relaxation (high T_{22}) in that time region for the two slow pH falls. This new and very interesting observation, namely, that the slower the development of rigor the later the breakage of cell membranes during rigor.

After 8 h p.-m. an extra-cellular space is even formed in the case of the two slow pH-drops (p_{21} starts to decrease) and this formation continues almost up to 7 days p.-m. We can also observe that there is a slow decrease of T_{21} after the breakage of the cell membranes. This type of behaviour of T_{21} has already been observed in the ageing of meat (Wahlgren and Tornberg, 1996). We suggested that this observation reflects a disordering of the myofibrillar structure, resulting in a shorter average distance a water molecule must travel before it encounters a protein surface; hence a shorter T_{21}. This elucidation of the T_{21} behaviour seems also to be applicable to the results presented in Figure 3a.

NIR

Figure 4 shows four MSC corrected NIR spectra for sample 01g12 measured 1.5, 5, 8, and 24 h p.-m. respectively. In the visible region there is a tendency that the NIR log(1/R) values decrease with time post-mortem. In the visual range of the NIR spectra two peaks of approximately same intensity are observed at 440nm and 568nm. Deoxymyoglobin has reported absorption maxima at 434nm (for the Soret band) and 555nm (Swatland, 1995). The Soret band for Oxymyoglobin is found at 416 nm, whereas the 555 nm peak splits up into two peaks at 542 and 578 nm wherefore the visual range of the reflectance spectra are dominated by the deoxymyoglobin. This is further substanciated by a very high correlation (r=0.94) between the NIR log(1/R) values measured at 440nm and 568nm indicating that the two peaks are caused by the same effect.

In the NIR region the spectra basically appear as that of water with major peaks at 970 nm (O-H strech, second overtone), 1450 nm (O-H strech, first overtone) and 1940 nm (O-H strech + O-H deformation). In NIR spectra protein information is usually found around 1500 nm (N-H strech, first overtone) and above approx. 2000 nm (combination bands).

However as evident from figure 4 if any such information is present in the spectra it is deep burried and extraction of any information requires sofisticated data analysis.

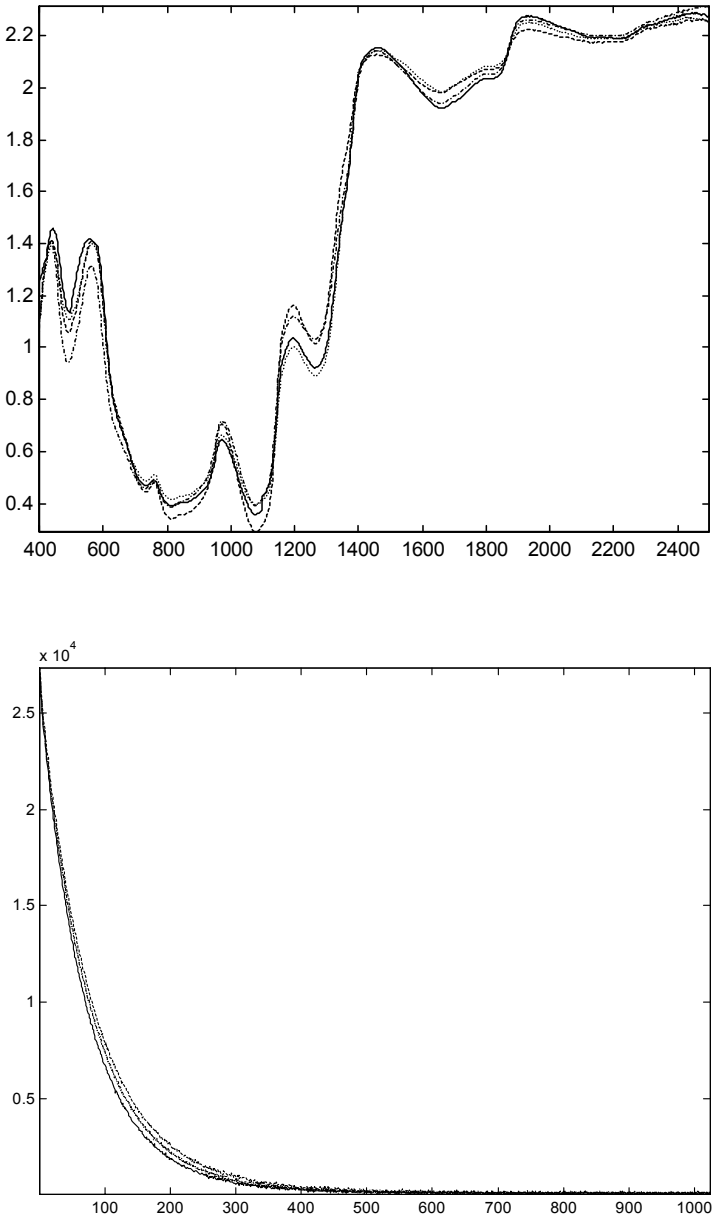

Figure 4. Top: NIR spectra (wavelength vs. 1/log(R)) of sample 01g20 measured at 1.5 (——), 3 (— —), 8 (---), and 24 (·—·) Hr p.-m (the spectra have been corrected by MSC). Below: NMR CPMG relaxation data (Tau vs. Intensity) of sample 03g12 measured at 1.5 Hr (—), 3 Hr (---), 8 Hr (·—·), og 24 Hr (— —).

COOKING LOSS, W-B SHEAR VALUES, AND MYOFIBRILLAR LENGTH AT FULLY DEVELOPED RIGOR

In Table 1 the instrumental recordings of tenderness (the inverse of W-B shear values), the proteolysis (Myofibrillar length) and cooking loss at fully developed rigor are collected. The only significant difference observed was between the fast and the other two groups with regard to the W-B shear measurements, where the fast pH course produced the most tender meat. This is in accordance with the observations of O'Halloran et al., (1997a).

Table 1. W-B shear values, Myofibrillar length and Cooking loss of the meat from the three pH groups at fully developed rigor(24h. p.-m.). Mean ±standard deviation is given.

pH group	*W-B shear values (N)*	*Myofibrillar length (µm)*	*Cooking loss (%)*
fast	120 ± 23	34± 10	29,2± 2,0
Medium	155± 32	35± 9	29,0± 2,5
Slow	164± 35	36± 9	29,1± 1,6

PRE-RIGOR RELATIONSHIPS AFTER 1.5 H P.-M.

A PCA was first performed on the NIR spectra and the complete NMR relaxation data separately. From the NMR data 4 significant components were found using full cross validation (or leave one out validation). These first four principal components described 97.98% of the data variance. For the NIR spectra the cross validated PCA showed 8 components to be optimal, which captured 99.61% of the variance in the total dataset. To simplify the interpretation of the further data analysis with the entire variable set, the score values from the initial PCA were used instead of the entire spectra. Thus, a PCA including 4 scores from the NMR relaxation curves (denoted NMR_1, NMR_2, etc.), 8 scores from the NIR spectra (denoted NIR_1, NIR_2, etc.), IT, SH, and pH was performed. The same procedure is followed at the succeeding time intervals. The score-plot of the analysis at 1.5 h p.-m. is shown in Figure 5a.

A relatively clear separation between the three pH-groups can be observed in the score-plot. The six samples with a slow pH-fall are lying in the right corner of the plot and are characterised by a high pH, SH and NMR_1 (Figure 5a and b). On the opposite side of the first PC, i.e. to the left side, the samples from the fast pH-group can be seen. They are characterised by high T_{21} values. At this early stage of rigor development astonishingly the shortening had proceeded to the greatest extent in the slow pH group (see Figure 2a and c) and also the lowest T_{21} is observed in this group. T_{21} is suggested to represent mostly the relaxation time of the protons within the fibre as discussed previously. It was suggested

that a higher intra-cellular protein concentration of the slow pH group may be one of the reasons for the lower T_{21} compared to the two faster pH drops.

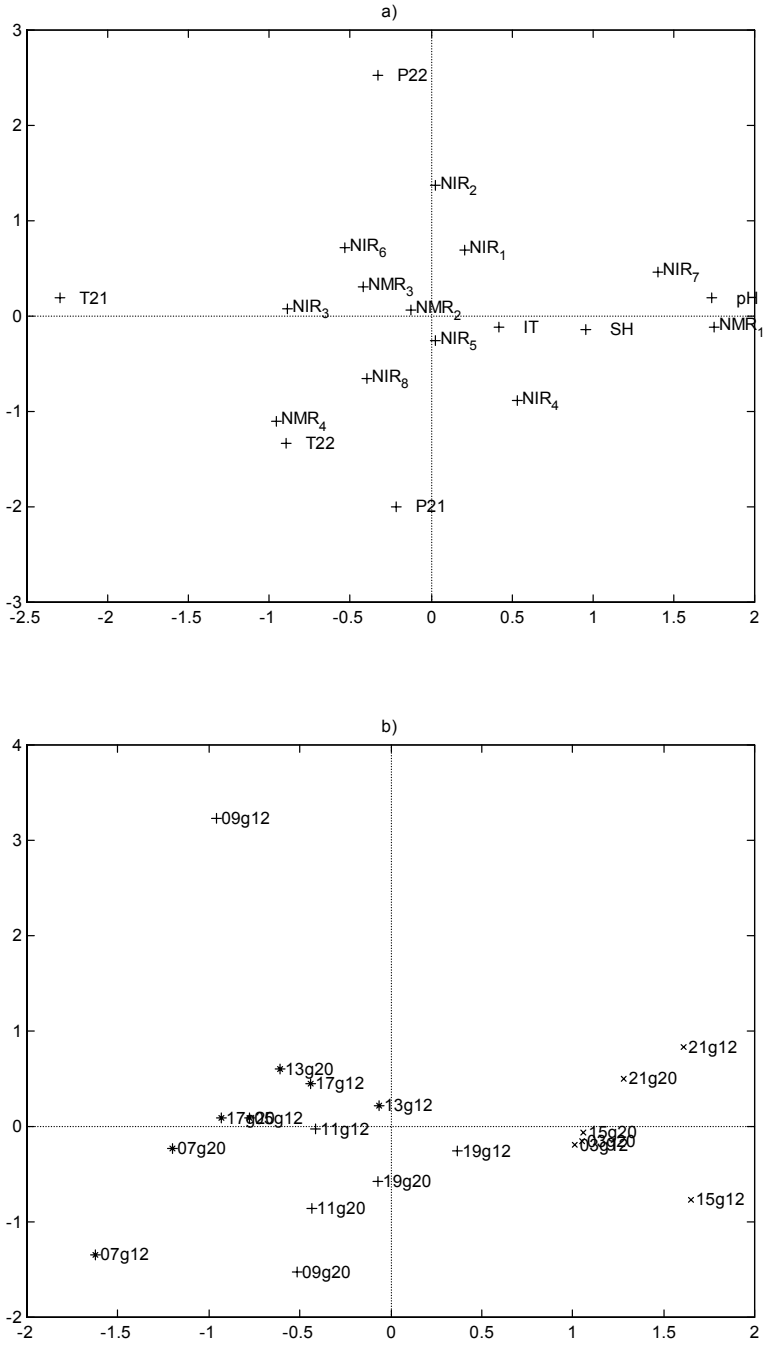

*Figure 5. Loading- (a) and scoreplots (b) of the two first principal components of a PCA-analysis of the NMR-, NIR-, pH-, IT- and SH-data obtained **1.5 hours** post-mortem. pH courses: *: fast; +: medium; x: slow. Percent variance captured by PC1 and PC2 was 38.5 %.*

The 2nd PC is characterised mainly by NMR-parameters, where a large relaxation time of the protons in the extra-cellular space (T_{22}) is especially characteristic for the medium pH group (Fig.5b). A high T_{22} is in turn related to a large number of protons within the myofibrillar space (p_{21}), which is in accordance with the evaluation given above, namely, that in the slower pH groups fewer or no membranes are destroyed at this early stage of the rigor development and that the commencing shortening most probably gives rise to a swelling of the fibres.

The NIR variables are all located close to the origo and therefore account for very little of the total variation. This indicates that the NIR data are of little information very early port mortem.

PRE-RIGOR RELATIONSHIPS AT 3H P.-M.

After 3 hours p.-m. there is still a good grouping of the three pH courses in the score-plot according to Figure 6b. The scores from the slow pH group are now in the first quadrant, and they are more spread over the plot. The degree of shortening (SH) and isometric tension (IT) still contribute to a large extent to the first principal component in the loading plot (Figure 6a), but the large change from 1.5 to 3 h p.-m. is that p_{21}, i.e. the number of protons in the intracellular space now governs the first PC more instead of PC2. This observation suggests that at this stage of the development of rigor the formation of an extra-cellular space starts to be important. p_{22} is the number of protons in the extra-cellular space and it is negatively correlated to p_{21}. This can be observed by the opposite location in the loading plot in Figure 6 a.

These two NMR variables determine most of the variation in PC1, which captures about 25 % of the variation in this data-set. According to the score plot (Figure 6b) the samples with the fastest pH drop are still mostly to the left on PC1, now characterised by a larger extra-cellular space than the other two groups.

It is also interesting to note that pH has shifted in the loading plot, now governing both PC1 and PC2 and still closely related to NMR_1. T_{21} now belongs to PC2 and is still negatively correlated to pH, probably for the same reason as suggested in the case of 1.5 hours p.-m. but now to a lesser extent. Furthermore, it can be observed among the samples in the medium pH-group that there is an outlier (09g12), which is more like the scores in the fast pH-group, having a larger extra-cellular volume (p_{22}).

Similar to the observations made at 1.5 h p.-m. the NIR information tends to be low. The variation in the spectra is low, and the score values are located close to the center of the scatterplot.

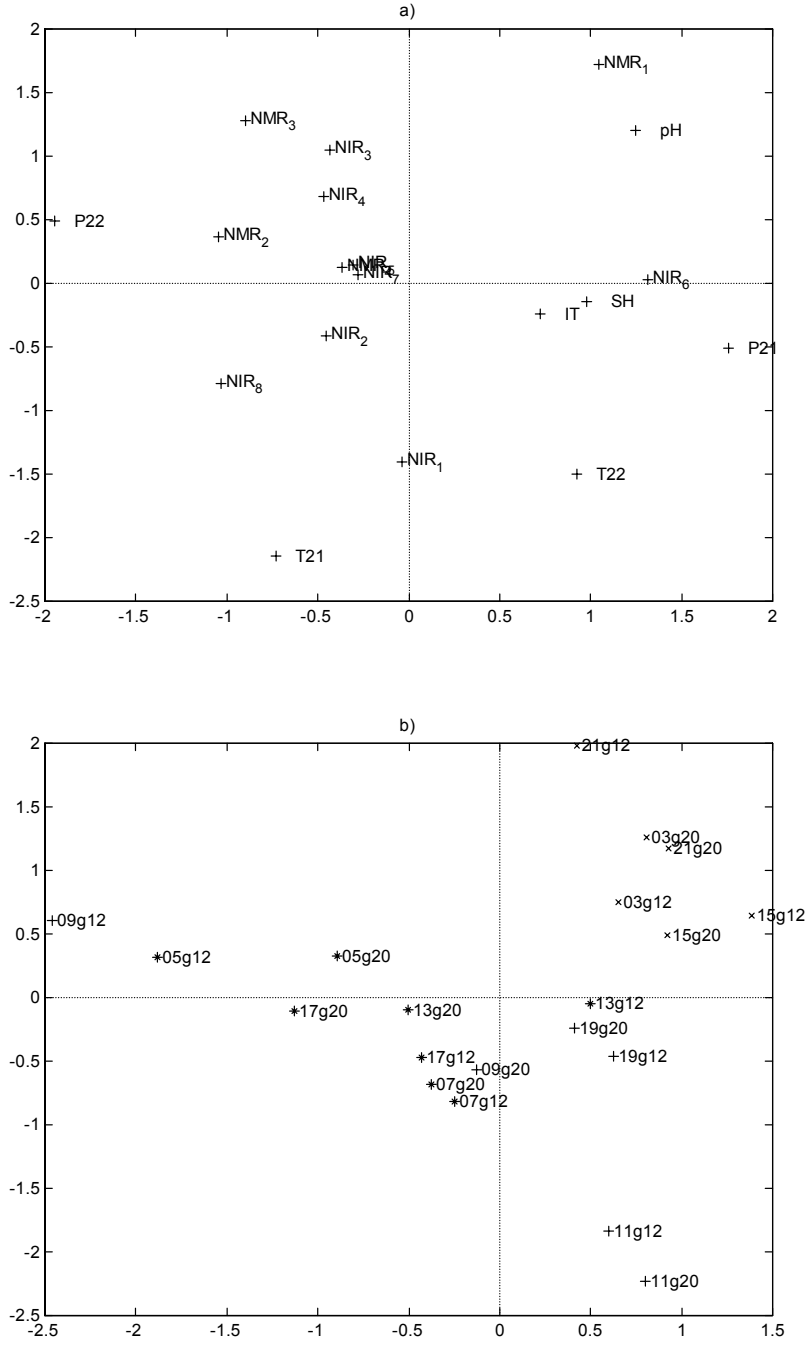

*Figure 6. Loading- (a) and scoreplots (b) of the two first principal components of a PCA-analysis of the NMR-, NIR-, pH-, IT- and SH-data obtained **3 hours** post-mortem. pH courses:; *: fast; +: medium; x: slow. Percent variance explained by the PC1 and PC2 was 42.7 %.*

PRE-RIGOR RELATIONSHIPS AT 5H P.-M.

The 5 hours loading- and score-plots can be seen in Figures 7a and b and the three pH groups are still discernible in the scoreplot. Now the scores from the medium pH-group are most frequent in the first quadrant, characterised by both a high T_{21} and T_{22}. This combination of the two relaxation times of the extra- and intra-cellular space suggests that the high T_{21} does not in this case imply a leakage of the sarcoplasmic proteins out into the extra-cellular space, but rather a swelling of the intracellular space due to shortening. That T_{22} is also high implies that the protein concentration is low in the extra-cellular space and consequently little protein has leaked out.

The scores of the fast pH-group are still found most frequently on the negative side of PC1, having a larger extra-cellular space than the two other groups. The samples from the slow pH group are all in the fourth quadrant. These scores are characterised by high pH and p_{21}, i.e. a large intra-cellular volume of protons and a swelled myofibrillar space. The influence of shortening and isometric tension has diminished compared to the former plots, as they are more centred around origo at this stage of the process.

PRE-RIGOR RELATIONSHIPS AT 8 H. P.-M.

The most frequent occurrence of the scores of the slow pH group is still in the fourth quadrant 8 hours post-mortem(Figures 8 a and b). They are also characterised by a high pH, p_{21} and NMR_1. According to Figure 3b the number of protons in the intra-cellular space is similar to that at the beginning of the rigor process in this slow pH group. This means that little extra-cellular space has been formed in these samples. Although shortening has occurred in these samples it creates more lateral swelling of the intra-cellular space (higher T_{21}) than disruption of the membranes and the formation of an extra-cellular space. This is, however, more the case for the fast pH group, where the scores are still on the left in the scoreplot. The outlier in the medium pH-group (09g12) from 5 h p.-m. has now merged more closely with the other scores in the same pH group.

The medium pH group is now at its peak of myofibrillar swelling according to Figure 3 and is therefore characterised by a high p_{21} and T_{21}, which place the scores predominantly in the first quadrant. The biggest change in the loading plot compared to 5 hours is that the isometric tension is more important for PC2. This means that the rigor process is now more pronounced.

Another interesting observation is that the PCs obtained from the NIR spectra are more prominent in PC2, especially NIR_5, being positively correlated to IT. This is an indication that the NIR information, which was low in the previous sampling intervals has increased at this stage of the rigor process.

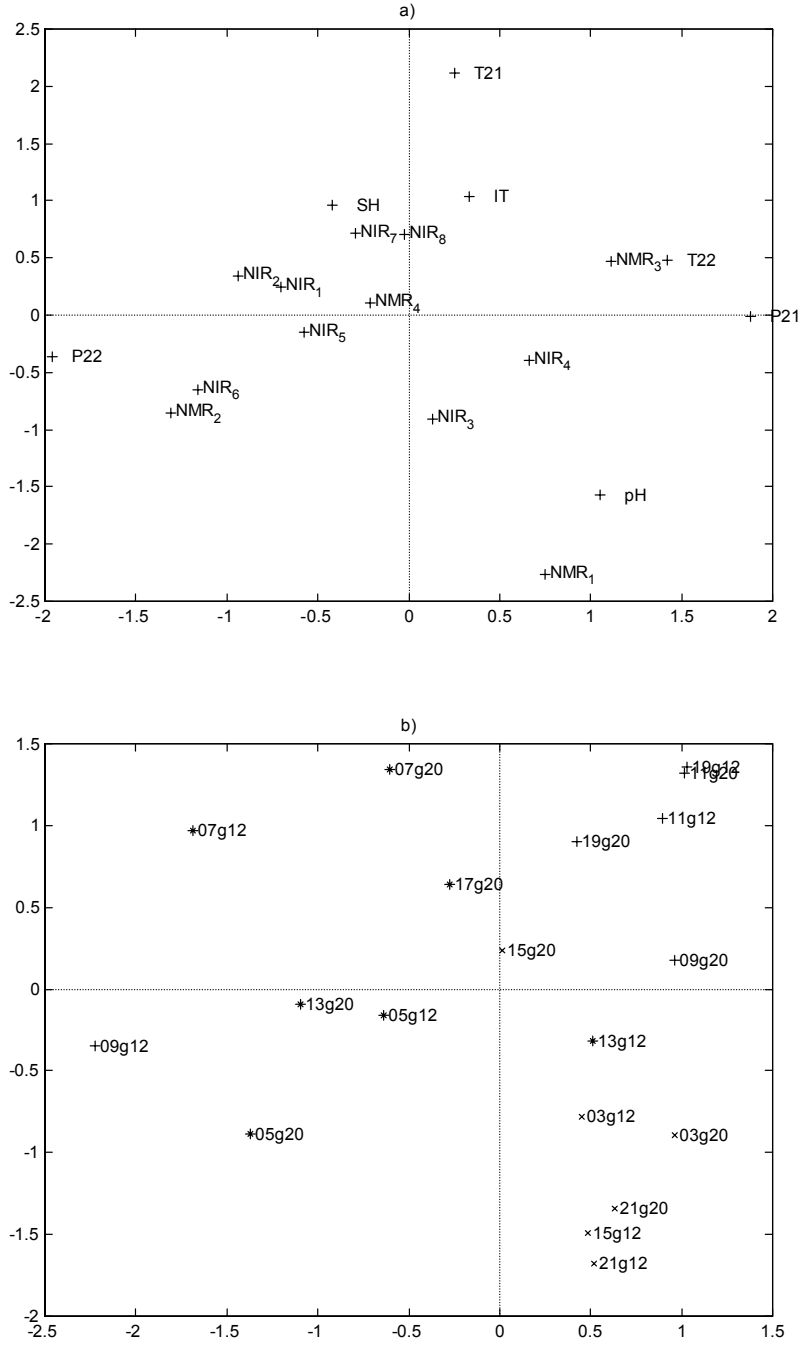

*Figure 7. Loading- (a) and scoreplots (b) of the two first principal components of a PCA-analysis of the NMR-, NIR-, pH-, IT- and SH-data obtained **5 hours** post-mortem. Legends of the pH courses as in the former Figures. Percent variance explained by the PC1 and PC2 was 38.5 %.*

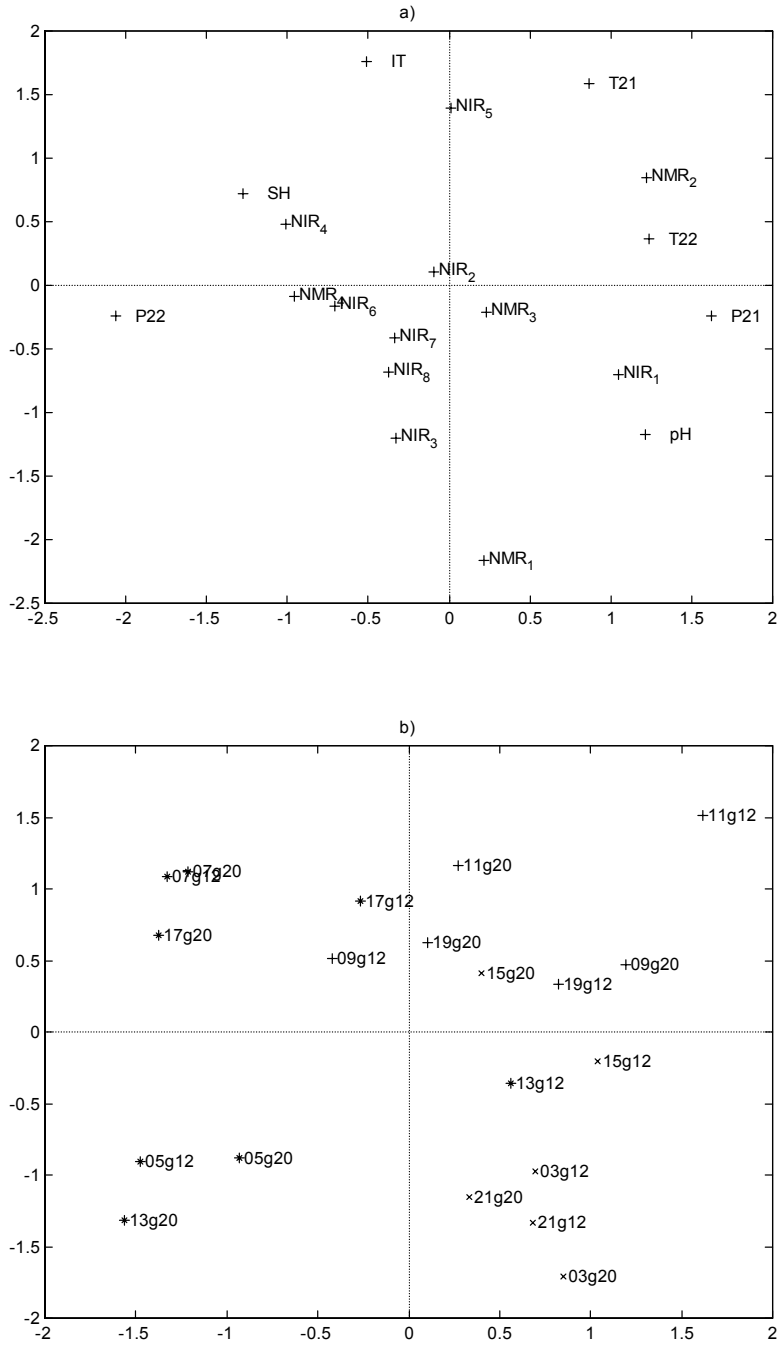

*Figure 8. Loading- (a) and scoreplots (b) of the two first principal components of a PCA-analysis of the NMR-, NIR-, pH-, IT- and SH-data obtained **8 hours** post-mortem. Legends of the pH courses as in the former Figures. Percent variance explained by the PC1 and PC2 was 41.2 %.*

PRE-RIGOR RELATIONSHIPS AT 24H P.-M.

Ultimately, the loading- and score-plots after 24 hours can be observed in Figure 9a and b. The separation of the scores into different pH groups are not so evident any longer, but trends are still to be seen. There are also changes in the loadingplot, where T_{21}, p_{21} and p_{22} explain most of the variation in PC1, and a high p_{22} correlates highly with a low T_{21} and p_{21}. These observations mean that the larger the extra-cellular space formed at fully developed rigor, the smaller is the T_{21}. This is contrary to the relationship found at the beginning of the rigor process, which can also be seen in Figure 3. The explanation of this behaviour may be that meat from the two slower pH drops have more time to swell laterally during rigor contraction, with less disruption of membranes leading to less formation of extra-cellular volume and leakage of sarcoplasmic proteins. This swelling of the myofibrillar space is then more important in lowering T_{21} than the loss in sarcoplasmic proteins from the intra-cellular space. Therefore the intra-cellular space is more swelled at fully developed rigor for the two slower pH drops than for the fast one. Most of the scores in the fast pH group also have a larger extra-cellular volume (high p_{22}). The slow and the medium pH groups are no longer separated in the scoreplot for 24 hours.

Moreover, T_{22}, IT, NIR_5 and pH explain most of the variation in PC2, where the shift in T_{21} and pH are the most remarkable compared to the plot at 8 h p.-m. After 24 hours NIR_5 and IT are still positively correlated. The degree of SH at fully developed rigor has now little influence on the loadings according to Figure 9a.

POST-RIGOR RELATIONSHIPS AT 24 H P.-M.

If the meat quality characteristics as measured after 24 hours, such as the W-B shear force, MFL, sarcomere length and cooking loss, are also involved in the PCA analysis, loading- and score-plots as in Figure 10 a and b are produced. According to the loading plot PC1 is mostly determined by p_{22} and T_{21}. MFL also explains the variation in PC1 and is inversely related to p_{22}. In the score plot the samples with a fast pH drop are still grouped predominantly on the left-hand side of the plot. They are characterised by a high extra-cellular volume and a low MFL. On the right-hand-side of the score-plot most of the results for the samples in the medium and the slow pH group are to be found. They can be described as having a relatively swollen intra-cellular space (high T_{21} and p_{21}) and a large MFL. The second PC is mostly explained by cooking losses, which are inversely related to NMR_2 and directly related to sarcomere length, pH and W-B shear force. A close relation is again observed between NMR_1 and pH. Evidently, at this early stage of the ageing process the W-B shear value is not only governed by MFL but also by the degree of shortening, here measured as sarcomere length.

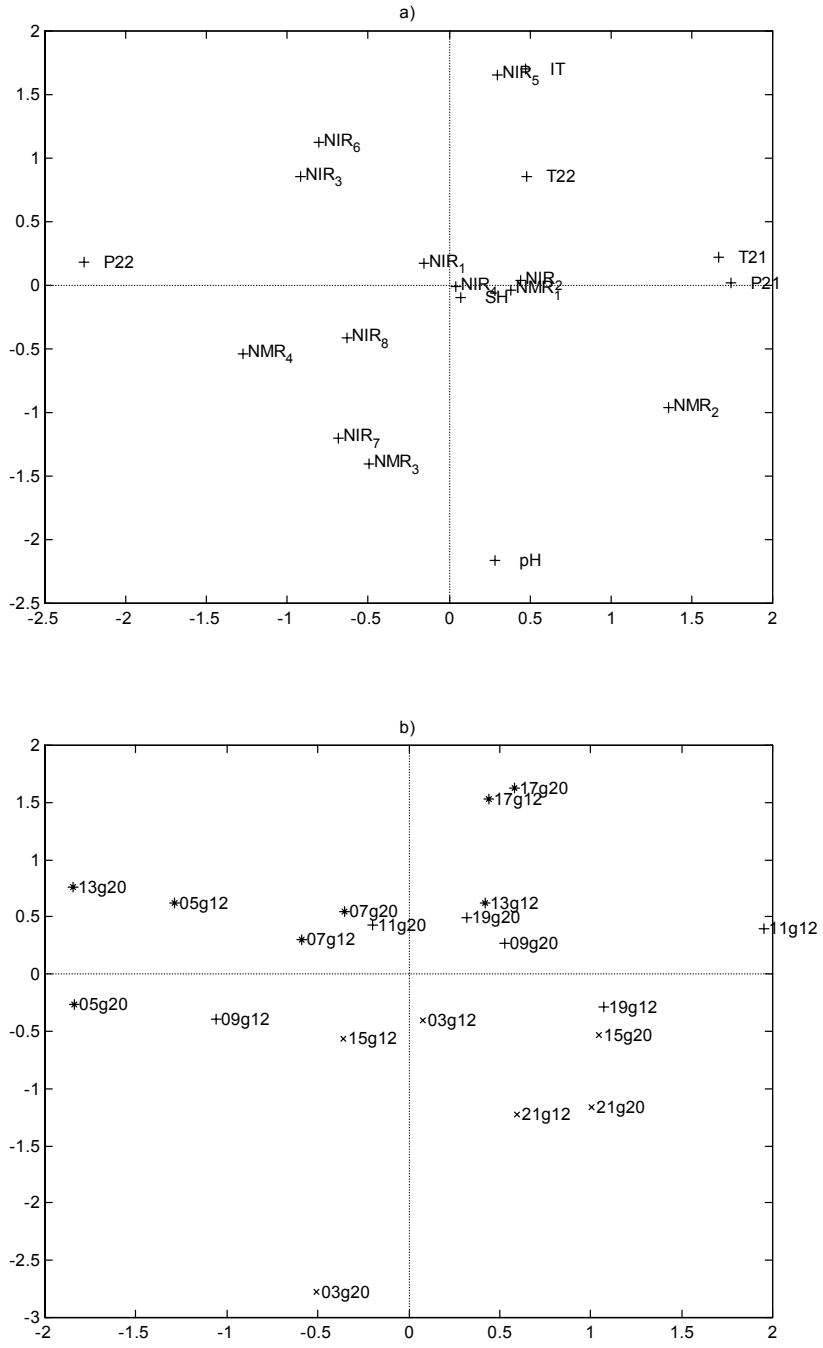

*Figure 9. Loading- (a) and scoreplots (b) of the two first principal components of a PCA-analysis of the NMR-, NIR-, pH-, IT- and SH-data obtained **24 hours** post-mortem. Legends of the pH courses as in the former Figures. Percent variance explained by the PC1 and PC2 was 33.5 %.*

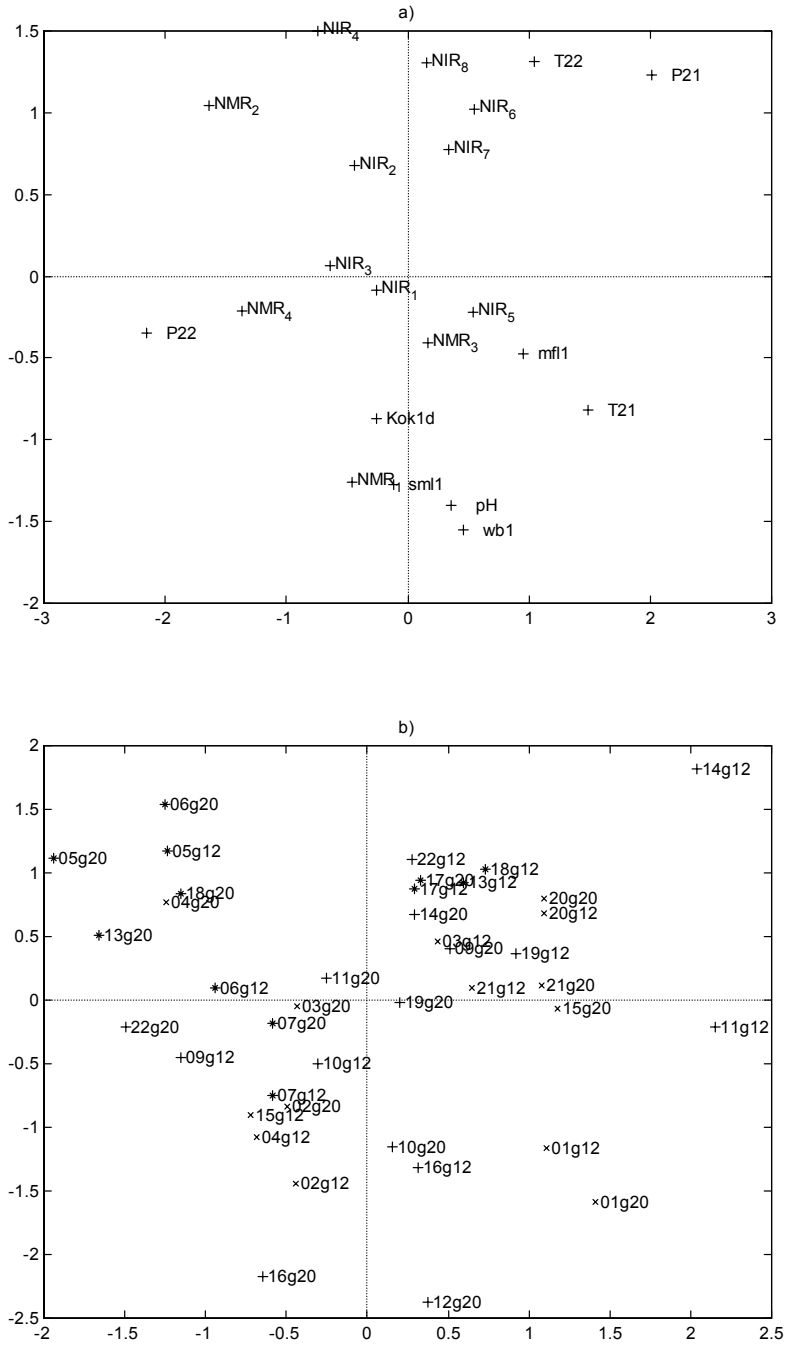

*Figure 10. Loading- (a) and scoreplots (b) of the two first principal components of a PCA-analysis of the NMR-, NIR-, pH-, sarcomere length (sml1)-, W-B shear force (wb1)-, cooking loss (kok1d)- and MFL-data obtained **24 hours** post-mortem. Legends of the pH courses as in former Figures. Percent variance explained by the PC1 and PC2 was 34.2 %.*

These results then suggest that the fastest glycolysing muscle (pH 5.6, 4-5 h) gives the most tender meat, i.e. the lowest W-B shear value. This is in accordance with the results of O'Halloran et al.,(1997), in which they found for that fast glycolysing LD muscles were rated more tender both in sensory and texture assessment, whereas slow glycolysing muscles were significantly tougher. At two days p.-m. they also found that the slow glycolysing muscles had shorter sarcomere lengths, which is also evident from our results as presented in Figures 10 a and b. However, the longer the ageing proceeds the more important the proteolysis becomes and O'Halloran et al., (1997), concluded that variation in proteolysis was the major cause of the difference in tenderness between slow, intermediate and fast glycolysing muscle.

The reason for this behaviour has been further investigated by the same group (O'Halloran, 1997b), and in their studies they found a higher overall activity of the calpains in the fast glycolysing muscle, which was explained by a higher activity of the μ-calpains and a lower calpastatin activity.

Tornberg, 1996, also suggested that the main tenderising effect, using ES, is based on the same mechanism as suggested in this investigation, i.e. the earlier that lower pH is attained in the rigor process the higher is the probability that the calpastatin is released from the μ-calpain at an earlier stage of the process. Thereby a greater proteolysis is achieved.

The NIR information is still very insignificant similar to the previous observations. In the current material no relation between the NIR signal, which is expected to originate in scatter effect owing to the muscle contraction, and the remaining measurements made have been found.

CONCLUSIONS

Beef LD muscles were subjected to three different pH drops during rigor (*fast:* pH 5.6, 4-5h p.-m.; *medium*: pH 5.6, 12h p.-m.*; slow*: pH 5.6, 20 h p.-m.) combined with two chilling regimes (20°C, 5h p.-m.; 12°C, 5h p.-m.) each. NMR-, NIR-, pH-, SH- and IT-measurements were used to characterise the meat during the development of rigor.

The fastest pH drop created larger extra-cellular volumes and cell membranes were destroyed, giving rise to a leakage of sarcoplasmic proteins at an earlier stage of the rigor process. This behaviour occurred later in the rigor process in meat subjected to the slower pH drop. These evaluations were based mainly on NMR measurements.

The fast pH group gave the most tender meat at fully developed rigor and measured as W-B shear values, although shortening was here among the highest. The reason for the superior tenderness of the fast pH group is suggested to be caused by a quicker and a more substantial proteolytic break-down for this type of meat compared to the more slowly glycolysing muscles.

The multivariate studies of the spectroscopic data revealed close relation between the NMR_1 and pH. Indications of an inverse correlation between the cooking loss and W-B values was observed. NIR spectroscopy revealed very little variation and no consequent relation to the physico-chemical parameters.

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