ELSEVIER

Food Chemistry 69 (2000) 407–418

Food Chemistry

www.elsevier.com/locate/foodchem

Pre-rigor conditions in beef under varying temperature- and pH-falls studied with rigometer, NMR and NIR

E. Tornberg [a,*], M. Wahlgren [a,1], J. Brøndum [b,c], S.B. Engelsen [b]

[a] *Swedish Meats R & D, PO Box 504, 224 24 Kävlinge, Sweden*
[b] *Food Technology, The Royal Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg, Denmark*
[c] *SFK Technology, Transformervej 9, 2730 Herlev, Denmark*

Abstract

Beef, *M. longissimus dorsi* (LD), were subjected to three different glycolytic rates during rigor (*fast:* pH 5.6, 4–5 h; *medium*: pH 5.6, 12 h*; slow*: pH 5.6, 20 h) combined with two chilling regimes (20°C, 5 h; 12°C, 5 h), in a 3∗2 factorial experimental design with three replicates. Spectroscopic techniques, such as pulsed low-field 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. Based mainly on interpretation of the NMR measurements it is demonstrated that meat with the fastest pH drop during rigor created larger extracellular volumes and cell membranes were destroyed, giving rise to a leakage of sarcoplasmic proteins at an earlier stage of the rigor process compared to meat subjected to a slower pH drop. The fast pH group yielded the most tender meat, measured as W–B shear values, at fully developed rigor, although shortening was among the highest for this group. The reason for the superior tenderness of the fast pH group is suggested to be caused by a quicker and a more substantial proteolytic breakdown for this type of meat compared to the more slowly glycolysing muscles. The multivariate studies of the spectroscopic data revealed a close relationship between the NMR measurements and pH. Indications of an inverse correlation between cooking loss and W–B values was observed. NIR spectroscopy revealed only little variation and no consequent relation to the physico-chemical parameters

1. 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 of being able to measure, predict and control, especially under processing conditions.

Rigor is a process that takes place post-mortem (p.-m.) and the understanding of this process is of vital importance, when trying to predict ultimate tenderness of meat in the early stages of the slaughtering process. During rigor, shortening of muscle fibres occurs and it is detrimental to tenderness. However, during and after rigor a concurrent, proteolytic degradation of the meat proteins also takes place, which is favourable for meat tenderness. The degree of shortening and proteolytic breakdown of muscle proteins is dependent not only on chilling conditions, but also on the stress ante-mortem and the course of pH prior to fully developed rigor.

When studying rigor development of *M. sternomandibularis*, Locker and Hagyard (1963) found that there exists both cold- and warm-shortening below and above 15 and 20°C. This work has been extended to cover more commercially relevant muscles (Devine, Wahlgren & Tornberg, 1999; Hertzman, Olsson & Tornberg, 1993; Honikel, Roncales & Hamm, 1983; Olsson, Hertzman & Tornberg, 1994). In three of these studies a rigometer, capable of recording shortening (S) as well as isometric tension (IT) during the development of rigor was used. Based on a study of S and IT during rigor it was suggested by Wahlgren, Olsson and Tornberg, (1997) that both the muscle shortening and the proteolytic degradation occurring during the rigor process could be measured.

These studies on constant temperatures during the development of rigor were followed by a study of the influence of different temperature-time courses on S, IT and tenderness in beef LD (Wahlgren, Olsson & Tornberg, 1997). The different chilling regimes studied

* Corresponding author at current address: Lund University, Department of Food Engineering, PO Box 124, 22100 Lund, Sweden.
E-mail address: eva.tornberg@livstek.lth.se (E. Tornberg).
[1] Present address: Norway Meat, PO Box 360 Okern, 0513, Oslo, Norway.

0308-8146/00/$ - see front matter
PII: S0308-8146(00)00053-4

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

Ultimately, different rates of 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 considerably from animal to animal (Kauffman & Marsh, 1987; O'Halloran, Troy & Buckley, 1997). One way to control the rate of glycolysis is to electrically stimulate (ES) at different times after slaughter. Chrystall, Devine and Davey (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 an early attainment of low pH at elevated temperatures (>15°C) can also 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, Devine and Tornberg (1997) where the medium pH-time course (pH 5.6 , 10 h p.-m.) yielded the best tenderness 3 days p.-m. in comparison with the quickest pH fall (pH 5.6, 1 h p.-m.). This phenomenon seems to be a plausible explanation for an optimum in pH decline often observed during rigor in relation to the ultimate tenderness of the meat, especially in the early stages of the ageing process (Geesink, van Laack, Barnier & Smulders, 1994; Marsh, Ringkob, Russell, Swartz & Pagel, 1987; Shackelford, Koohmaraie & Savell, 1994; Takahashi, Lochnert & Marsh, 1984). 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 is that of O'Halloran, Troy and Buckley (1997). They found that the fastest glycolysing muscle (pH 5.6, 6 h p-m). resulted in 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-declines (12 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 studies by O'Halloran, Troy and Buckley (1997) the muscles were divided into different pH-groups according to their natural variation in glycolytic rates. Moreover, in the present study a number of methods were used to characterize the rigor process more thoroughly. In addition to 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) 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, instrumental and sensory texture in both raw and processed meat is the use of the non-invasive NMR (Brøndum, Byrne, Bak, Bertelsen & Engelsen, 1999; Fjelkner-Modig & Tornberg, 1986). The multi-exponential decay of the transverse relaxation time, T_2, of water protons in muscle, especially pork muscle, has been reported abundantly in the literature (Borisova & Oreshkin, 1992; Fjelkner-Modig & Tornberg, 1986; Larsson & Tornberg, 1988; Renou, Kopp, Gatellier, Monin & Kozak-Reiss, 1989; Renou, Monin & Sellier, 1985). Two dominating, discernible relaxation processes are generally observed, where the major fraction (80%) of the muscle water has a T_2 of 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 holding the 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, Clark & Jones, 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 become a well-established method for rapid quantitative analysis of foods (Osburne & Fearne, 1986), but the ability of NIR to predict quality parameters in foods such as the tenderness of beef, has also been explored (Hildrum, Nilsen, Mielnik & Naes, 1994; Park, Chen, Hruschka, Shackelford & Koohmaraie, 1998). However, the predictability of NIR measurements for tenderness of meat has not yet been studied at the early stages of the post-mortem period, i.e. during the rigor process. Results from such measurements will be presented in this paper.

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 of the spectral data. The most important chemometric techniques for use in meat quality have

recently been reviewed by Næs, Baardseth, Helgesen and Isaksson, (1996).

2. 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 (80 V, 15 Hz, 5 ms, 48 s);
(3) low voltage electrical stimulation applied 30 min post-mortem (80 V, 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 h 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 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 h p.-m.) 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 approximately 35 mm long and weighing 1.5–2 g, longitudinally oriented along the fibre axis, 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 at an accuracy of ± 0.5°C.

Small pieces of muscle (approximately 1–2 g) were cut out at selected time intervals (1.5, 3, 5, 8 and 24 h p.-m.) and subjected to NMR and NIR measurements. NMR measurements 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, 1024 data points in total. Sixteen acquisitions were accumulated with a repetition time of 4 s. The CPMG relaxation curves were fitted both to a one-component exponential model and a two-component exponential model according to the equation:

$$M(t) = \Sigma_i p_{2i} \cdot \exp\left[-t/T_{2,i}\right] + B$$

where $M(t)$ is the magnetisation amplitude at time t. p_{2i} are the fractions of water within the meat in different environments, while T_{2i} are the corresponding characteristic transverse relaxation times. A three component model was judged as an overfitting of the recorded data. The NMR relaxation data was separately subjected to a principal component analysis (PCA) (see Bechmann, Pedersen, Nørgaard & Engelsen, 1999).

NIR measurements were made with a NIR Systems Inc. model 6500 dispersive instrument (Silver Springs, Maryland, USA), covering the wavelengths from 400 to 2498 nm (thus, also including the visual region). At every second 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 and 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 were subsequently scatter corrected using multiplicative scatter correction (MSC) prior to PCA (Geladi, McDougall & Martens, 1985).

After fully developed rigor (24 h p.m.) samples were cut into 1.5 cm thick slices and fried on a frying pan to an internal temperature of 70°C. Loss of weight or cooking loss was determined as the percentage weight loss during 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 (3 cm) and myofibrillar length (MFL) (10 g). The Warner–Bratzler shear force measurements were performed using an Instron® Universal testing machine (4301) equipped with a modified Warner–Bratzler shear blade with a square opening of 26×21 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 in ice to room temperature. The maximum shear force for at least 10 pieces (0.7×1.5 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 & Tornberg, 1992). A small sample of *Longissimus* muscle (about 5 g) was cut into small pieces using a knife. Fifty milliliters 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 1000 *g*. After discharge of the supernatant, 25 ml of I-medium were added to the sediment, and the samples were brought into suspension. One milliliter 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 (×1340) and 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 (Sony 3 CCD) and an image analysing programme, the Image Pro Plus 3.0 (Media Cybernetic, USA) on single fibres prepared by fixing in 2% glutaraldehyde and carefully torn 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 relationships between spectral and chemical data were analysed using principal component analysis (PCA) (Martens & Næs, 1993). All multivariate data studies were performed in Unscrambler 6.2 (CAMO, Trondheim, Norway) and Matlab 5.2 (Mathworks Inc., Natick, MA, USA).

3. Results and discussion

3.1. pH and temperature fall

The two chilling regimes were linear up to 5 h p.-m., when the slower reached 20°C and the quicker 12°C (Fig. 1a). From 5 to 12 h the linear time-temperature slope diminished, reaching the same temperature of about 9°C at 12 h for both chilling regimes.

The different types of electrical stimulation gave essentially three different rates of pH fall during rigor development (Fig. 1b). The three pH-time courses, *fast* (reaching pH 5.6 after 4–5 h p.-m.), *medium* (reaching pH 5.6 after 12 h p.-m.) and *slow* (reaching pH 5.6 after 20 h p.-m.) 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 (Fig. 1b), however, the differences were not significant.

3.2. Muscle shortening and isometric tension

The development of rigor at the two temperature falls and the three pH-falls can be observed in Fig. 2a–d. As revealed by the figure, the degree of shortening never exceeded 25%. A large animal to animal variation was sometimes observed. However, using ANOVA a significant difference was observed in shortening at the

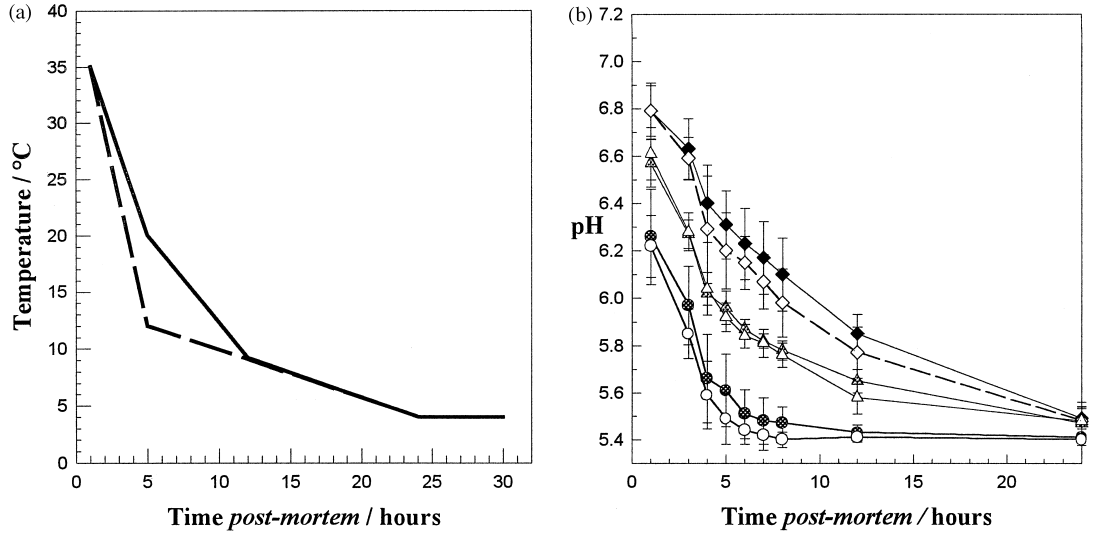

Fig. 1. (Left) The two chilling regimes used in the experiment. (Right) The pH-fall as a function of time post-mortem for the *fast* (●, ○) *medium* (▲, △) and *slow* (◆, ◇) pH-courses. Open symbols are slow chilling (20°C, 5 h) and filled symbols fast chilling (12°C, 5 h).

slowest temperature fall (20°C, 5 h), where the medium pH drop gave rise to least shortening compared to the slow and the fast drops.

The ranking order with regard to maximum IT was observed to be the same for the three pH courses at 20°C, 5 h as well as at 12°C, 5 h. This means that the meat with the fastest pH drop achieved the highest maximum IT, the medium pH drop obtained the medium IT and the slowest pH fall had the lowest IT.

For muscle shortening, the same ranking order for the three pH courses at the two different temperature falls was observed. 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.

Generally the amount 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, Wahlgren & Tornberg, 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 can be explained by the fact that the development of rigor is quicker in the fast pH drop group and there is, therefore, a higher probability for more fibres to go into rigor at a higher temperature and to achieve a larger degree of warm-shortening.

3.3. NMR

The NMR measurements showed that during early post mortem the 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 relaxation curve better. We, therefore, used a two-component system to decompose the NMR relaxation data. The fast component had a relaxation time (T_{21}) varying from 40 to 52 ms (Fig. 3a), which is in accordance with earlier observations. This was also the case for the slower

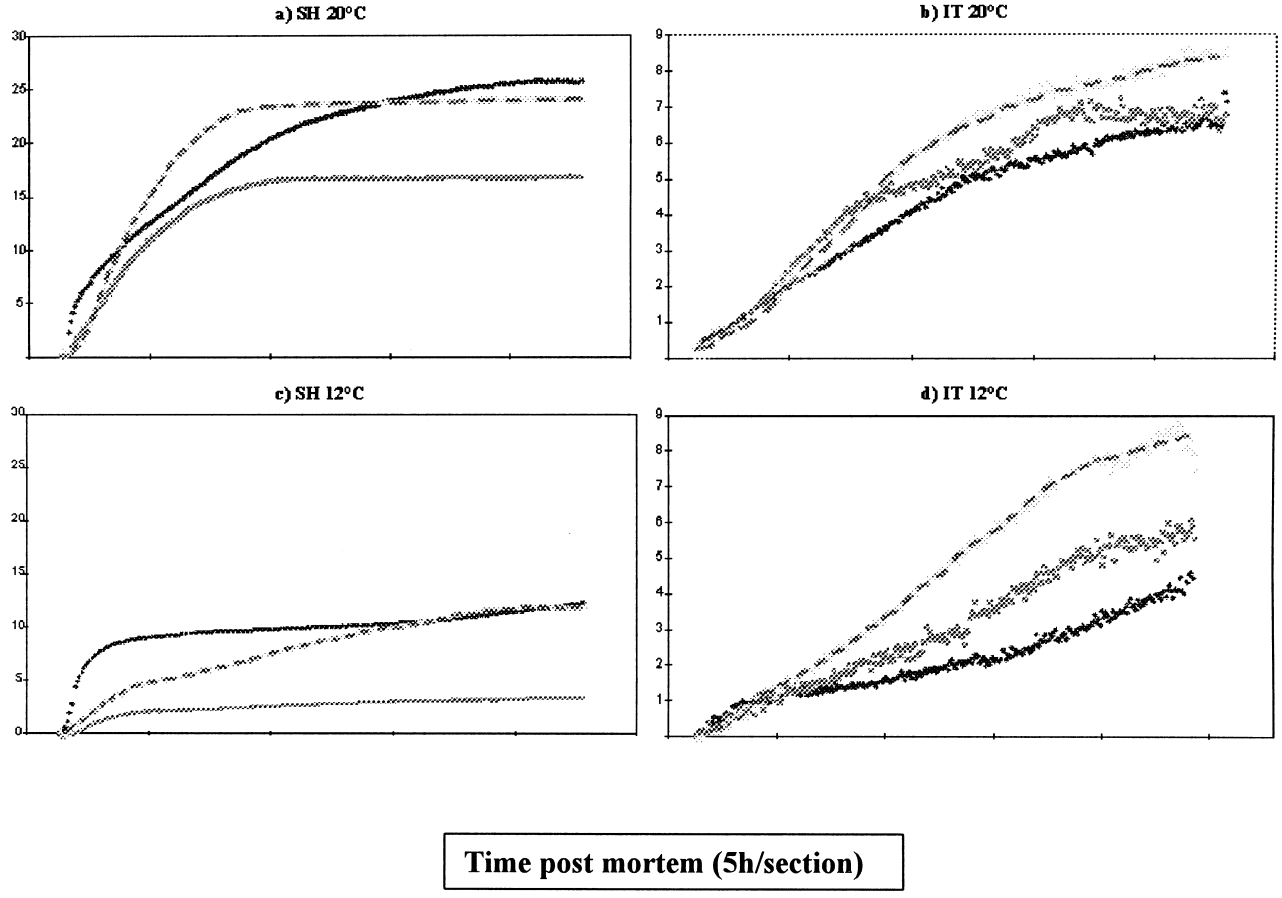

Fig. 2. The course of shortening SH (%) (a, c) and isometric tension IT (kPa) (b, d) during rigor development at a temperature fall of 20 (a, b) and 12°C (c, d) after 5 h. *Slow* (black dots), *medium* (grey dots) and *fast* (— —) represent the three different pH drops studied, i.e. *slow*: pH 5.6 after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

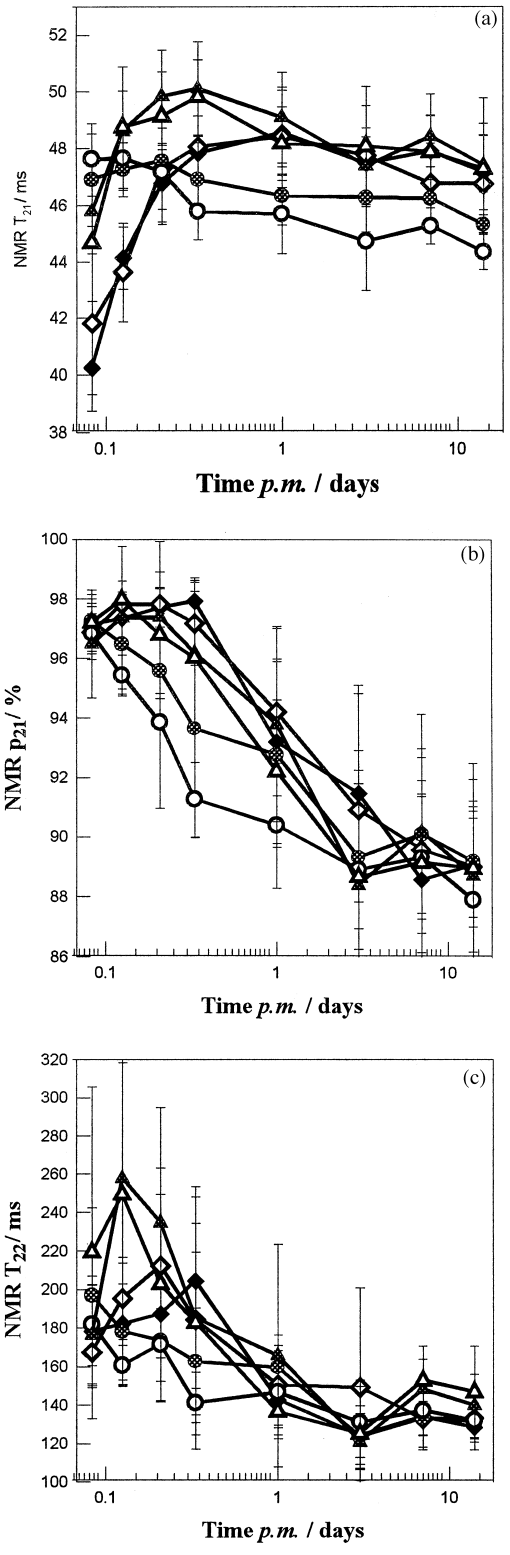

Fig. 3. (a) T_{21} as a function of time post-mortem, pH-time and temperature-time regime during rigor and ageing. Legends as in Fig. 1.(b) p_{21} as a function of time post-mortem, pH-time and temperature-time regimes during rigor and ageing. Legends as in Fig. 1.(c) T_{22} as a function of time post-mortem,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

relaxing component (T_{22}) which had a variation in the range 100–300 ms (Fig. 3c). The number of protons, p_{21}, relaxing according to the quickest relaxation time (86–100%) is somewhat higher compared to results given in literature in which only post-rigor meat has been analysed (Fig. 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 (Fig. 3a). The group with a *fast* rigor development, however, started with a relatively fast relaxation time and 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 which a decrease was observed (Fig. 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 generally 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. It is well-established that during rigor the cell membranes are partly destroyed and the quicker the rigor the faster the leakage of the sarcoplasmic proteins. Tornberg, Andersson, Gøransson and von Seth (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 relationship 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.

With this reasoning in mind the time-courses of the NMR-data shown in Fig. 3 can be further evaluated. If T_{21} is then assumed to be the relaxation time for the protons within the fibre, a high T_{21} indicates a lower protein concentration. This can be achieved either by the cell membranes being more disrupted and hence more sarcoplasmic proteins leaking out in the extra-cellular space or the fibres are more swollen. We know that during rigor the cell membranes are partly disrupted and the quicker the rigor the faster the leakage of the sarcoplasmic proteins. As displayed in Fig. 3a, T_{21} decreases with prolonged time post-mortem in the case of the fast pH fall, whereas for the other two pH groups T_{21} increases up to about 8 h p.-m. It can be assumed that swelling of the myofibrillar space takes place during

this period which probably is due to a longitudinal contraction (Fig. 2) under isovolumetric conditions occurring in the early stages of the rigor process. That the cell membranes are still intact up to 8 h p.-m. and, therefore, no leakage of the sarcoplasmic proteins has occurred is corroborated by the high content of protons in the myofibrillar space (p_{21}) and the slow relaxation time in the extra-cellular space (high T_{22}) during that time interval for the two slow pH falls. This is a 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. more extra-cellular water is being generated even in the case of the two slow pH-drops (p_{21} starts to decrease) and this trend continues up to almost 7 days p.-m. One can also observe that there is a minor decrease in T_{21} after the breakage of the cell membranes. This T_{21} behaviour has already been observed in the ageing of meat (Wahlgren & Tornberg, 1996). We suggested that this observation reflects a disordering of the myofibrillar structure due to the proteolytic action, resulting in a shorter average distance a water molecule must diffuse before it encounters a boundary, hence a shorter T_{21}. This elucidation of the T_{21} behaviour seems also to be applicable to the results presented in Fig. 3a.

3.4. NIR

Fig. 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 for the NIR $\log(1/R)$ values to decrease with time post-mortem. In the visual range of the NIR spectra two peaks of approximately the same intensity are observed at 440 and 568 nm. Deoxymyoglobin has reported absorption maxima at 434 nm (for the Soret band) and 555 nm. 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, so the visual range of the reflectance spectra is dominated by the deoxymyoglobin. This is further substantiated by a very high correlation ($r = 0.94$) between the NIR $\log(1/R)$ values measured at 440 and 568 nm, 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 stretch, second overtone), 1450 nm (O–H stretch, first overtone) and 1940 nm (O–H stretch + O–H deformation). In NIR spectra protein information is usually found around 1500 nm (N–H stretch, first overtone) and above approx. 2000 nm (combination bands). However, as evident from Fig. 4, if any such information is present in the spectra, it is buried deeply and extraction of any information requires sophisticated data analysis.

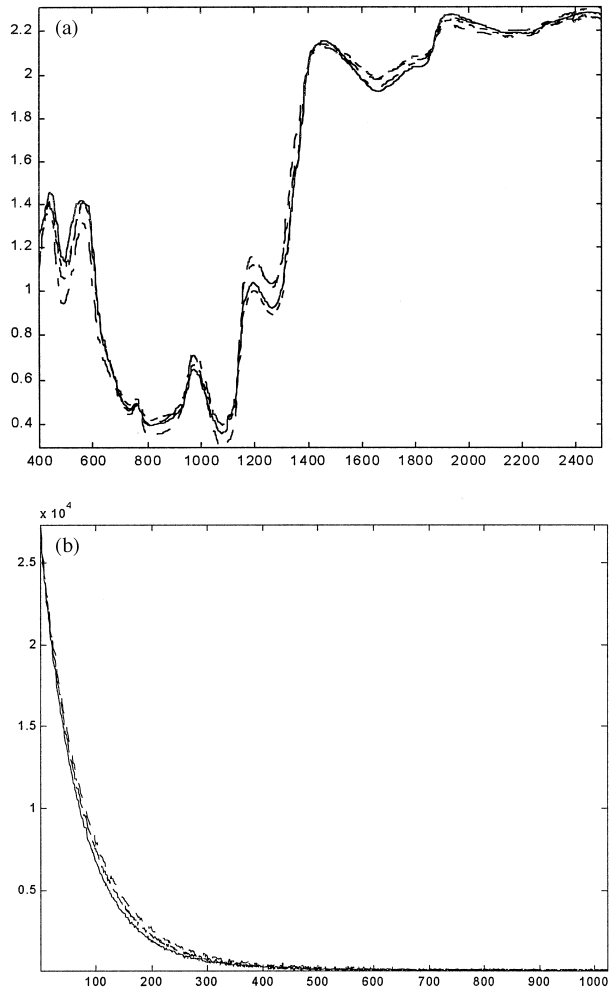

Fig. 4. (a) NIR spectra of sample 01g20 measured at 1.5 (—), 3 (— —), 8 (- - -), and 24 (· — ·) h p.-m. (the spectra have been corrected by MSC). (b) A NMR CPMG relaxation data of sample 03g12 measured at 1.5 (—), 3 (- - -), 8 (· — ·), and 24 h (— —).

3.5. 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 were collected. The only significant difference observed was between the fast and the other two pH 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, Troy and Buckley (1997).

3.6. Pre-rigor relationships after 1.5 h p.-m.

From the principal component analysis (PCA) of the NMR relaxation curves 4 significant components were found using full cross validation (leave one out validation). These first four principal components described

Table 1
W–B shear values, myofibrillar length and cooking loss of the meat from the three pH groups at fully developed rigor (24 h p.-m.)[a]

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

[a] Mean ± standard deviation is given.

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 data-set. 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, another 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 was followed at the succeeding time intervals. The score-plot of this second PCA analysis at 1.5 h p.-m. is shown in Fig. 5a, where the two principal components shown capture 38.5% of the variation in the material.

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

The second 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 in 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.

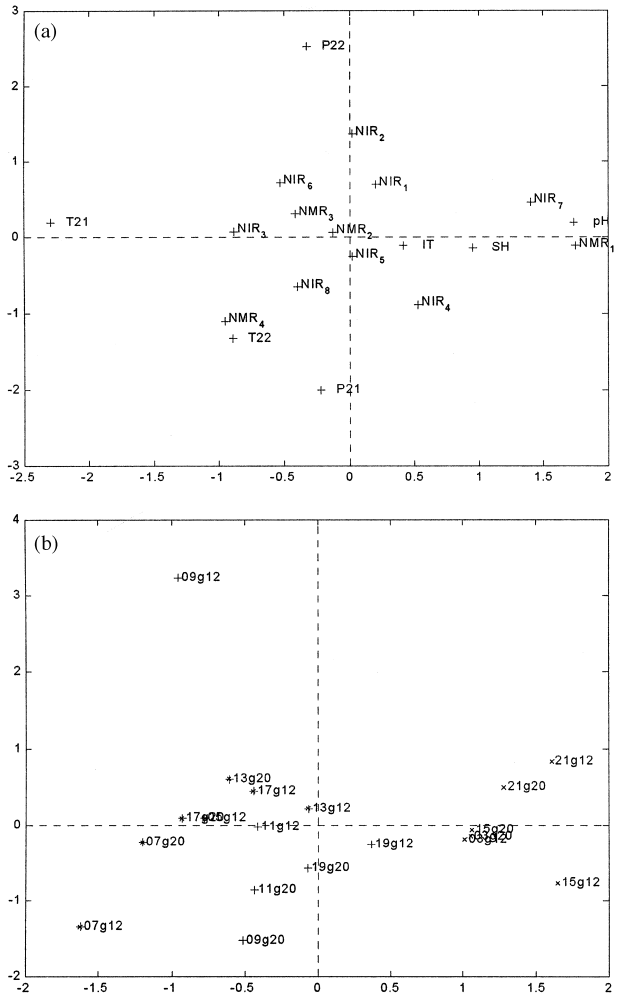

Fig. 5. Loading- (a) and scoreplots (b) of the two first principal components of a PCA-analysis of the NMR, NIR, pH, IT and SH data obtained 1.5 h post-mortem. pH courses: *: *fast*; +: *medium*; x: *slow*. Percentage variance captured by PC1 and PC2 was 38.5%.

This indicates that the NIR data contribute with little information very early post mortem.

3.7. 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 (Fig. 6a and b). They are also characterised by a high pH, p_{21} and NMR_1. According to Fig. 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 only little additional extra-cellular water 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 more extra-cellular water. This is, however, more the case for the fast

pH group, where the scores are still to the left in the score-plot. The outlier in the *medium* pH-group (09g12) from 1.5 h p.-m. has now merged more closely with the other scores in the same pH group.

According to Fig. 3, the *medium* pH group is now at its peak of myofibrillar swelling and is, therefore, characterised by a high p_{21} and T_{21}, which places the scores predominantly in the first quadrant. Compared to 1.5 h, now the isometric tension is more important for PC2 than for PC1, as in the former figure. This means that the rigor process is now more pronounced.

Another interesting observation is that the PC's 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, has increased at this stage of the rigor process.

3.8. Pre-rigor relationships at 24 h p.-m.

Ultimately, the loading- and score-plots after 24 h can be observed in Fig. 7a and b. The separation of the scores into different pH groups is not so evident any longer, but trends are still to be seen. There are also changes in the loading plot, 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 Fig. 3. The explanation of this behaviour may be that meat from the two slower pH drops has 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

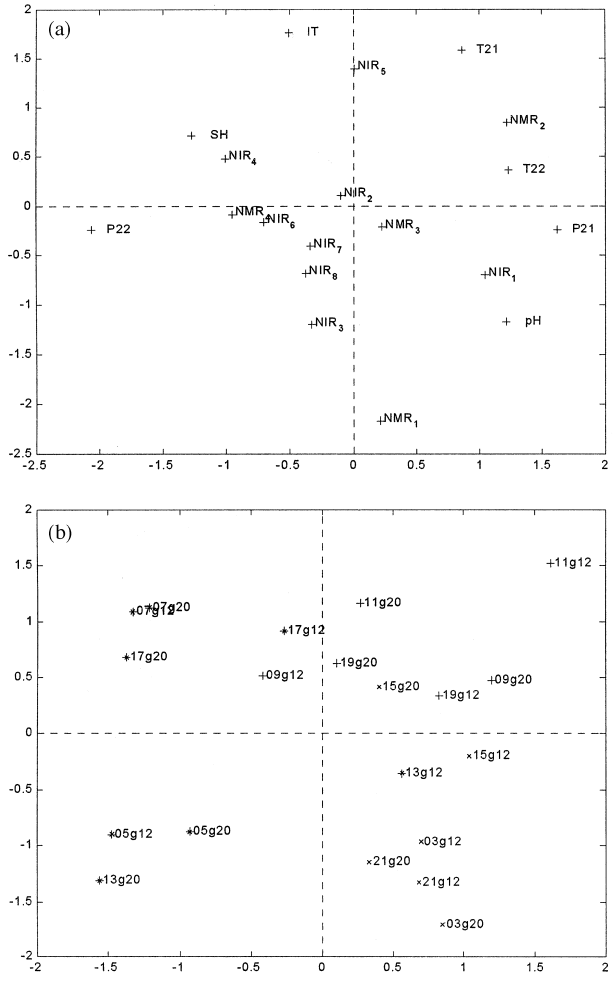

Fig. 6. Loading- (a) and scoreplots (b) of the two first principal components of a PCA-analysis of the NMR-, NIR-, pH-, IT- and SH-data obtained 8 h post-mortem. Legends of the pH courses as in the former figures. Percentage variance explained by the PC1 and PC2 was 41.2%.

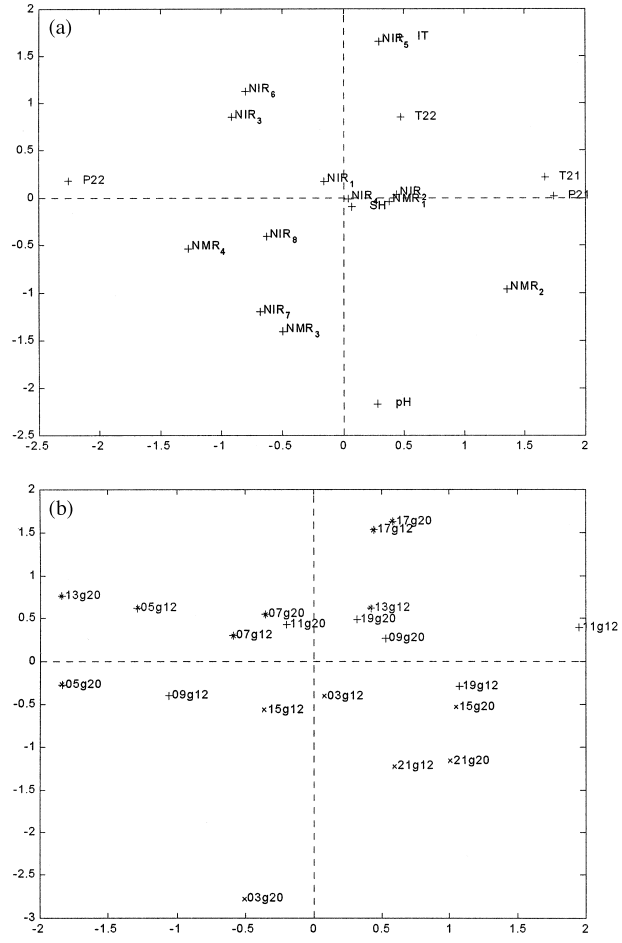

Fig. 7. Loading- (a) and scoreplots (b) of the two first principal components of a PCA-analysis of the NMR-, NIR-, pH-, IT- and SH-data obtained 24 h post-mortem. Legends of the pH courses as in the former figures. Percentage variance explained by the PC1 and PC2 was 33.5%.

more important in lowering T_{21} at this stage of the rigor process 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 score plot for 24 h.

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 events compared to the plot at 8 h p.-m. After 24 h 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 Fig.7a.

3.9. Post-rigor relationships at 24 h p.-m.

If the meat quality characteristics as measured after 24 h, 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 Fig. 8a 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. Most of the results for the samples in the medium and the slow pH group are to be found on the right-hand-side of the score-plot. 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 relationship is again observed between NMR_1 and pH. Evidently, at this early stage of the aging process the W–B shear value is not only governed by MFL but also by the degree of shortening, measured here as sarcomere length.

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, Troy and Buckley (1997) in which they found that the 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 Fig. 8a and b. However, the longer the aging progresses the more important the proteolysis becomes and O'Halloran, Troy and Buckley (1997) concluded that variation in proteolysis was the major cause of the difference in tenderness between slow, intermediate and fast glycolysing muscle.

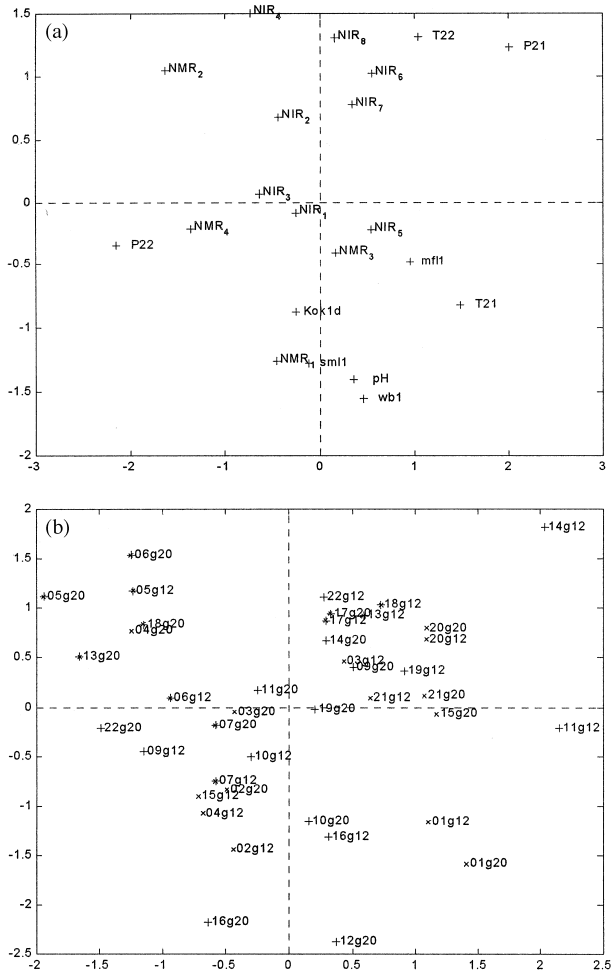

Fig. 8. Loading- (a) and scoreplots (b) of the two first principal components of a PCA-analysis of the NMR-, NIR-, pH-, sarcomere length (sml1)-, W–B shear force (wb1)-, cooking loss (kok1d)- and MFL(mfl1)-data obtained 24 h post-mortem. Legends of the pH courses as in former figures. Percentage variance explained by the PC1 and PC2 was 34.2%.

The reason for this behaviour has been further investigated by the same group (O'Halloran, Troy, Buckley & Reville, 1997). 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. This means that the earlier in the rigor process a low pH is attained the higher the probability that the inhibitor calpastatin is released from the μ-calpain at an earlier stage of the process and thereby a greater proteolysis is achieved.

The NIR information is still very insignificant, as in 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 was found.

4. Conclusions

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

Meat with the fastest pH drop during rigor 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, compared to meat subjected to a slower pH drop. These evaluations were based mainly on NMR measurements.

The fast pH group gave the most tender meat at fully developed rigor, measured as W–B shear values, although shortening was among the highest in this group. The reason for the superior tenderness of the fast pH group is suggested to be caused by a quicker and a more substantial proteolytic breakdown for this type of meat compared to the more slowly glycolysing muscles. The multivariate studies of the spectroscopic data revealed a close relationship 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 relationship to the physico-chemical parameters.

Acknowledgements

This work was funded by the EU project FAIR-PL96-1107 as well as the Scan organisation, Sweden. The authors also appreciate the skilful technical assistance of Ms Lena Sjöberg. J. Brøndum was supported by ATV and S. B. Engelsen was supported by funds from The Danish Research Councils (SJVF) and from The Danish Food Research Centre for Advanced Food Studies (LMC). Finally we are much indepted to Professor Lars Munck for bringing the groups together and to Gilda Kischinovsky for help with the manuscript.

References

Bechmann, I. E., Pedersen, H. T., Nørgaard, L. and Engelsen, S. B. (1999). Comparative chemometric analysis of transverse low-field ^{1}H NMR relaxation data, In: P. S. Belton, B. P. Hills & G. A.Webb. *Advances in magnetic resonance in food science*. Royal Society of Chemistry, (pp. 217–225).

Borisova, M. A., & Oreshkin, E. F. (1992). On the water condition in pork meat. *Meat Science, 31*, 257–265.

Brøndum, J., Byrne, D. V., Bak, L. S., Bertelsen, G., & Engelsen, S. B. (1999). Warmed-over flavour in porcine meat — a combined spectroscopic, sensory and chemometric study. *Meat Science, 54*, 83–95.

Chrystall, B. B., Devine, C. E., & Davey, L. (1980). Studies in electrical stimulation: post-mortem decline in nervous response in lambs. *Meat Science, 4*, 69.

Devine, C. E., Wahlgren, N. M., & Tornberg, E. (1999). Effect of rigor temperature on muscle shortening and tenderisation of restrained and unrestrained beef *m. longissimus thoracicus* et *lumborum*. *Meat Science, 51*, 61–72.

Fjelkner-Modig, S., & Tornberg, E. (1986). Water distribution in Porcine *M. longissimus dorsi* in relation to sensory properties. *Meat Science, 17*, 213–231.

Geesink, G. H., Van Laack, R. L. J. M., Barnier, V. M. H., & Smulders, F. J. M. (1994). Does electrical stimulation affect the speed of ageing or ageing response? *Sciences Des Aliments, 14*, 409–422.

Geladi, P., McDougall, D., & Martens, H. (1985). Linearization and scatter-correction for near-infrared reflectance spectra of meat. *Applied Spectroscopy, 39*, 491–500.

Hertzman, C., Olsson, U., & Tornberg, E. (1993). The influence of high temperature, type of muscle and electrical stimulation on the course of rigor, ageing and tenderness of beef muscle. *Meat Science, 35*, 119–141.

Hildrum, K. I., Nilsen, B. N., Mielnik, M., & Naes, T. (1994). Prediction of sensory characteristics of beef by near-infrared spectroscopy. *Meat Science, 38*, 67–80.

Honikel, K. O., Roncales, P., & Hamm, R. (1983). The influence of temperature on shortening and rigor onset in beef muscles. *Meat Science, 8*, 221–241.

Kauffman, R. G. & Marsh, B. B. (1987). In J. F Price and B. S. Scheigert. *The science of meat and meat products*. (3rd ed), (pp. 349). Westport, CT: Food and Nutrition Press.

Larsson, G. & Tornberg, E. (1988). An attempt to relate meat quality of pork (*M. longissimus dorsi*) to meat structure. In *Proceedings of the 34th International Congress of Meat Science and Technology*, Brisbane. (pp. 588).

Lillford, P. J., Clark, A. H. & Jones, D. V. (1980). Distribution of water in heterogeneous food and model systems. In: S. P. Rowland, *Water in polymers, ACS Symposium series* (No. 127, pp. 177).

Locker, R. H., & Hagyard, C. J. (1963). A cold shortening effect in beef muscles. *Journal of the Science of Food and Agriculture, 14*, 787–793.

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

Martens, H., & Naes, T. (1993). *Multivariate calibration* (2nd ed.). New York: Wiley.

Næs, T, Baardseth, P., Helgesen, H., & Isaksson, T. (1996). Multivariate techniques in the analysis of meat quality. *Meat Science, 43*, 135–149.

O'Halloran, G. R., Troy, D. J., & Buckley, D. J. (1997). The relationship between early postmortem pH measurements and the tenderisation process of beef muscle. *Meat Science, 45*, 239–251.

O'Halloran, G. R., Troy, D. J., Buckley, D. J., & Reville, W. J. (1997). The role of endogenous Proteases in the tenderisation of fast glycolysing muscle. *Meat Science, 47*, 187–210.

Olsson, U., Hertzman, C., & Tornberg, E. (1994). The influence of low temperature, type of muscle and electrical stimulation on the course of rigor, ageing and tenderness of beef muscles. *Meat Science, 37*, 115–131.

Olsson, U., and Tornberg, E., (1992) The interrelationship between myofibrill fragmentation and tenderness for beef meat. *Proceedings of the 38th International Congress of Meat Science and Technology*, vol. 3. (pp. 399–402). Clermont Ferrand, France.

Olsson, U., Wahlgren, N. M., & Tornberg, E. (1995). The influence of pre-slaughter stress on muscle shortening, isometric tension and meat tenderness of beef. *Proceedings of the 41st International Congress of Meat Science and Technology*, (pp. 614). San Antonio.

Osburne, B. G., & Fearne, T. (1986). *Near infrared spectroscopy in food analysis*. Harlow, Essex: Longman Sci. & Tech.

Park, B., Chen, Y. R., Hruschka, W. R., Shackelford, S. D., & Koohmaraie, M. (1998). Near infrared reflectance analysis for predicting beef longissimus tenderness. *Journal of Animal Science, 76*, 2115–2120.

Renou, J. P., Kopp, J., Gatellier, Ph, Monin, G., & Kozak-Reiss, G. (1989). NMR-relaxation of water protons in normal and malignant hypothermia-susceptible pig muscle. *Meat Science, 26*, 101–114.

Renou, J. P., Monin, G., & Sellier, P. (1985). Nuclear magnetic resonance measurements on pork of various qualities. *Meat Science, 15*, 225–233.

Shackelford, S. D., Koohmaraie, M., & Savell, J. W. (1994). Evaluation of *Longissimus dorsi* muscle pH at three hours post mortem as a predictor of beef tenderness. *Meat Science, 37*, 195–204.

Takahashi, G., Lochnert, J. V., & Marsh, B. B. (1984). Effects of low-frequency electrical stimulation on beef tenderness. *Meat Science, 11*, 207–225.

Tornberg, E. (1996). Biophysical aspects of meat tenderness. *Meat Science, 43*, 175–191.

Tornberg, E. & Larsson, G. (1986). Changes in water distribution of beef muscle during cooking — as measured by pulse NMR. *Proceedings of the 32nd European Meeting of Meat Research Workers*, (pp. 437). Ghent.

Tornberg, E., Andersson, A., Göransson, Å., & von Seth, G. (1993). Water and fat distribution in pork in relation to sensory properties. In E. Poulanne & D. I. Demeyer, *Pork quality: genetic and metabolic factors*. (pp. 239–258). CAB International.

Wahlgren, N. M., Devine, C. E., & Tornberg, E. (1997). The influence of different pH-courses during rigor development on beef tenderness. *Proceedings of the 43rd International Congress of Meat Science and Technology GI-37* (pp. 622–623). Auckland, New Zealand.

Wahlgren, N. M., Olsson, U., & Tornberg, E. (1997). The influence of different temperature-time courses on muscle shortening and beef tenderness. *Proceedings of the 43rd International Congress of Meat Science and Technology* 27 July to 1 August (pp. 624–625). Auckland, New Zealand.

Wahlgren, N. M., & Tornberg, E. (1996). Ageing of beef studied by using different instrumental techniques and sensory tenderness. *Proceedings of the 42nd International Congress of Meat Science and Technology*, Lillehammer, Norway.