Session 3 Meat quality

L 2 PROTON PULSE NMR MEASUREMENTS IN BEEF AND PORK IN RELATION TO THE QUALITY TRAITS

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INTRODUCTION

Nuclear Magnetic Resonance (NMR) offers a way to non-invasively and non-destructively study small molecules in the meat per se. Different nuclei have been studied. Using so called high resolution NMR with high magnetic fields ^{31}P- NMR gives for example the possibility to follow the decay of high-energy phophate compounds during rigor development (Vogel et.al., 1985) and to study the hydrolysis of added phosphates in chicken meat (Belton et.al., 1987). Another nucleus that has been studied is ^{23}Na, following the ingress of sodium ions into post rigor porcine muscle during brining (Guiheneuf et. al., 1997). However, using low magnetic field (< 0.5 T) NMR instruments the most abundant used nucleus to study is ^{1}H or the proton.With such a low-field bench-top NMR instrument nowadays both the water and fat content of flesh can be measured in the same sample, using a gradient magnetic field (Jepsen, et.al., 1999). A relatively new development of NMR measurements is the magnetic resonance imaging (MRI), which gives a spatial resolution (about 300 μm) of fat, water and connective tissue in meat by proton-NMR (Laurent et.al., 2000, Bonny et.al., 2000).

In this review, however, will we concentrate on NMR measurements that are most easily related to the quality traits of meat and it has been shown that the most abundantly used measurement in this context is the bulk proton pulse NMR.

ELUCIDATION OF PROTON PULSE NMR MEASUREMENTS

In a proton pulse NMR measurement a radio frequent pulse will transfer some of the protons of the water to a higher energy state, i.e. in a position against the applied magnetic field. The relaxation of those protons, going back to the lower energy position, being parallel to the magnetic field, is registered, using low-field pulse NMR measurements. There are two types of relaxation times, namely spin-lattice or longitudinal(T1) and transversal or spin-spin (T2) relaxation times. As the last relaxation time, T2, has a much larger variation and therefore give more information it has been measured more frequently. Only this type of proton relaxation will be discussed further on.

The elucidation of T2 measurements of the water in biological tissue and biopolymer system like meat has been under debate since the early 1970s. Water protons in meat have shorter transverse relaxation times (mainly T2 about 40 ms) than in bulk water (2 s) and the reason for that was early suggested to be some long-range ordering of the water in the tissue. Hills et.al., 1996 have convincingly demonstrated that this is not the case for biopolymer systems and biological tissues. It is usually sufficient to consider only three states of water, namely 'structural and bound water', surface water and bulk water. Structural water is the water hydrogen bonded inside the groves and cavities of globular proteins. The surface water is what we usually call the hydration water of the macromolecule. This hydration water extends only one or two molecular layers form the surface of the bio-polymer. Bulk water is the rest of the water.

By assuming that the fast exchange between the small bound fraction of hydration and structural water and the larger capillary held or bulk water is the cause of the shorter T2-relaxation, a short T2 suggests a short diffusion distance of the bulk water to the exchange site. This means that the larger the protein concentration in the meat the lower the T2, because the diffusion distance to an exchange site for the water protons is shorter. However, it has been observed (Halle et al., 1981 and 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., 1989, is the cross-relaxation involving non-water protons in the protein molecules. These lowers the relaxation time of the structural and hydration water causing a smaller observed average T2. Total water content in a muscle is about 75 %. The amount of hydration water is around 5 % and the rest of the water is held by capillary forces.

Only one relaxation rate will be observed when the exchange of magnetisation between all the states of water is fast compared with the difference in relaxation rates. Multiple relaxation rates will be observed when this is not the case, as for example for whole meat. Two dominating, discernible relaxation processes of T2 are generally observed, where the major fraction (80%) of the muscle water has a T2 between 35-50 ms, whereas the remaining water relaxes in the range of 100-150 ms. Hills et.al., 1996 have shown for randomly packed beds of Silica and Sephadex particles that when the diffusion of water magnetisation between different pores or spatial regions in the sample is on the NMR relaxation time scale the relaxation becomes multiple exponential and reflects the pore size distribution and the distribution between the pores. This reasoning can easily be transferred to the meat system. Because the protein concentration is much higher inside the cell, due to the fact that both the myofilaments and the sarcoplasmic proteins are placed there, compared to the extra- cellular space, where only sarcoplasmic proteins exist, T2 is substantially shorter inside than outside the cell. Therefore the fraction of water relaxing with the shortest relaxation time can be considered as mainly intracellular water. The T2 relaxing process of 100-150 ms is then associated with the water in the extra-cellular space. The consequence of this behaviour is that the extra cellular and the intra cellular space can be studied, using proton pulse NMR, although the membrane does not act as a barrier for proton exchange. This type of interpretation has been confirmed by Tornberg et.al., 1993, where the diffusion controlled proton exchange in the extra cellular space was proved by using the Einstein equation according to: $T22 = x^2/2D$. The average diffusion time in this case is related to the slowest relaxation time T22 and that time plotted against the squared distance between fibre bundles, x^2, would result in a straight line if D, the diffusion constant, is constant. This was also the case as can be seen in Figure 1, obtaining a straight line with $r = 0.85^{***}$

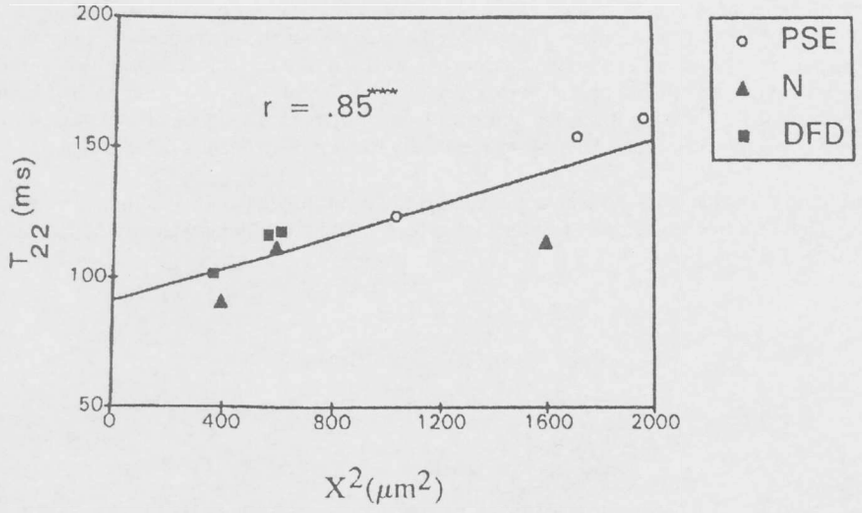

Figure 1: The slowest transverse relaxation time, T_{22}, in raw porcine LD muscle of different qualities (N, PSE, DFD) as a function of the squared distance, x^2, between fiber bundles. From Tornberg et. al., 1993.

MEASUREMENTS OF QUALITY TRAITS IN MEAT USING NMR

Important quality traits of whole pork and beef are tenderness/consistency, taste/flavour, appearance, juiciness and water-holding capacity. These properties of the meat are in turn dependent on the breeds used, pre-rigor and post-rigor conditions and on the cooking method. In this paper changes in the water distribution within the meat as a function of the above enumerated variables, using proton pulse-NMR and light microscopy, will be reviewed.

Breeds

The water distribution in muscle *longissimus dorsi* (LD), using proton pulse NMR, of purebred Hampshire and Yorkshire and of Large White, halothane negative and halothane positive Pietrain have been registered by Fjelkner-Modig and Tornberg, 1986 and Renou et.al., 1985, respectively. Essentially, two transverse relaxtion times of the water in the meat were observed in both the investigations. The shortest relaxation time, T21 of about 40 ms, was not significantly different with regard to breeds, whereas stressed animals gave rise to meat with lower T21 according to Renou et.al., 1985. The reason for the lower T21 could be an increased amount of structural water inside the cavities formed between aggregated proteins. Those are in turn created by the quick pH drop , caused by stress, during rigor with a concomitant cooling of the carcass that has not proceeded that far. Moreover, Renou et.al., 1985 observed that the amount of protons relaxing with the shortest relaxation time, i.e. the intra cellular water, was less for the stressed animals.

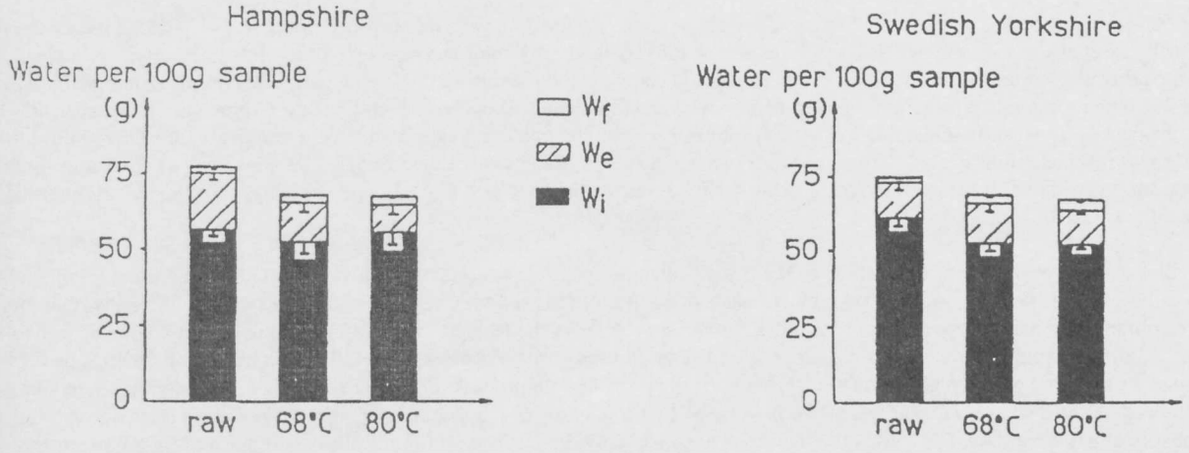

Figure 2: The proton population of free (W_f, having a relaxation time of 2 s), extra cellular (W_e) and intra cellular water (W_i) in *M. longissimus dorsi* from purebred Hampshire and Swedish Yorkshire, when raw, fried to a centre temperature of 68°C and 80°C, respectively. From Fjelkner-Modig et.al., 1986.

The situation was the same for Fjelkner-Modig and Tornberg, 1986, when comparing the raw meat from the Hampshire breed with the Yorkshire breed., i.e. the intra cellular water was less for the former breed than for the latter. When the intra cellular water was less by necessity the extra cellular water increased, which can be seen Figure 2, when comparing the raw meat of Hampshire and Swedish Yorkshire. The reason for this similarity in observation between the two investigations is, however, not the same, because in the former investigation the quick pH drop caused the differences observed, whereas in the latter study it was the ultimate pH that differed between the breeds. Meat from Hampshire had a lower ultimate pH and therefore a less swollen myofibrillar space and subsequently less water in the intra cellular space.

It can also be observed from Figure 2 that the extra-cellular water is larger in Hampshire than in Yorkshire, when raw, but is the other way around, when cooked to 80°C. These studies also showed that the higher content of intra-cellular water of the Hampshire meat, when cooked, gave.a more tender meat.

Rigor development

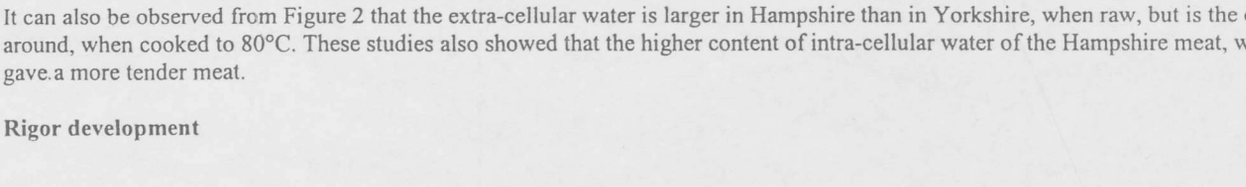

Figure 3: **a)**T_{21}, **b)**p_{21} and **c)** T_{22} as a function of time post-mortem, pH- and temperature-time regime during rigor and ageing. Three pH-time courses were used, *fast* (●, ○) (reaching pH 5.6 after 4-5 hours post-mortem), *medium* (▲,Δ) (reaching pH 5.6 after 12 hours post-mortem) and *slow* (◆,◇) (reaching pH 5.6 after 20 hours post-mortem). Filled symbols are slow chilling (20°C, 5h) and open symbols fast chilling (12°C, 5h). From Tornberg et.al., 2000.

Rigor is a process that takes place post-mortem and the understanding of this process is of vital importance, when trying to predict ultimate tenderness of meat. During rigor shortening of muscle fibres and proteolytic degradation of the meat proteins occur. In a study by Tornberg et.al., 2000 beef meat from *M. longissimus dorsi* was studied during rigor using three different 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) in combination with two different chilling regimes (20 and 12°C at 5h post mortem). At selected time intervals (1.5, 3, 5, 8 and 24 hours *post-mortem*) the muscles were subjected to NMR-measurements (the transverse relaxation time (T_2) of the water protons), using a Maran, bench-top pulsed NMR analyser. A two-component relaxation of T_2 was mostly observed.

In Figure 3 the fast component T_{21}, the slow component T_{22} and the number of protons, p_{21}, relaxing according to the quickest relaxation time can be seen as a function of time post-mortem, pH- and temperature-time regime during rigor and ageing. 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 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. 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.

A higher T_{21} indicates a lower protein concentration in the myofibrillar space. This suggests that either the cell membranes are less intact and sarcoplasmic proteins have leaked out in the extra-cellular space or the protein matrix within the fibre is more swollen. We know that during rigor the cell membranes are partly destroyed and the quicker the rigor the faster the leakage of the sarcoplasmic proteins. 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} then 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 a longitudinal contraction taking place under isovolumetric conditions at 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 suggested 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.

Based on these proton pulse NMR measurements on rigor development in beef meat we can state that the meat with the fastest pH drop creates larger extra-cellular volumes and cell membranes are 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.

Stress and water holding of meat

Animals can be subjected to both long-term and short-term stress. The former exhausts the animal and depletes the glycogen content and results in lower lactic acid production during glycolysis, hence a higher ultimate pH. This type of meat appears darker and when $pH_u > 6.2$ for beef muscle LD, it is called DFD (dark, firm and dry) meat. Stress just prior to slaughter may cause rapid glycolysis and a fast pH-drop. This in combination with a relatively high temperature, as the cooling of the carcass has not proceeded that far, results in denaturation and aggregation of the muscle proteins. This gives rise to PSE- meat (pale, soft and exudative). As the name of the latter type of meat tells us the water holding is less for PSE meat compared to normal (N) and DFD meat.

When comparing these three different qualities of pork, i.e. normal, PSE and DFD, using proton pulse NMR, the PSE-meat had the shortest relaxation time for the myofibrillar water and the DFD-meat had the shortest relaxation time for the extra-cellular water (Larsson and Tornberg, 1988; Tornberg et. al., 1993a; Tornberg et. al., 1993b). When comparisons were made with micrographs of the transverse sections of meat of these varying quality with the slowest relaxation time ,T_{22}, it was observed that the wider the channel around the fibre bundles the longer the T_{22} relaxation. This is the relationship visualised in Figure 1(Larsson and Tornberg, 1988; Tornberg et. al., 1993a). Offer et. al., 1989 have confirmed that the drip losses from meat predominantly arises from these longitudinal channels through the meat between the fibre bundles. Consequently, good correlations have been found between drip loss and T_{22} relaxation time (Tornberg et. al., 1993a, r = 0.60; Bröndum et.al., 2000, r = 0.72; Bertram et. al., 2001, r = 0.77)

Ageing

In a study by Wahlgren and Tornberg, 1996 the ageing of beef LD muscles was followed measuring the transverse relaxation time, using proton pulse NMR. Two groups of ultimate pH were compared, one with a normal $pH_u < 5.6$ and one with an increased pH_u ($5.8 < pH_u < 6.0$). The ageing procedures investigated were 3, 7, 14, and 21 days at + 4°C or 7, 14, 21, 35, 49, 63, and 77 days at – 1.5 °C. The T21, which was the fastest of the two relaxation times observed, and as we have discussed before can be considered as the water inside the myofibrils, was significantly decreased by ageing time except for meat at high ultimate pH and stored at + 4°C (Figure 4). This means that the average distance for a water molecule to a protein surface within the myofibrillar space decreases with prolonged ageing time.

From Figure 4 it can also be observed that there is a significant negative relationship between T21 and the sensory determined tenderness, i.e. a faster relaxation time is linked to a higher degree of tenderness. This might be explained by an increase in the unordered structure, due to the break down of myofibrillar structure on ageing. This causes a lowering in the T21 relaxation time, because the probability of a water molecule to hit a protein surface and thereby resulting in an exchange is higher in a more unordered protein structure.

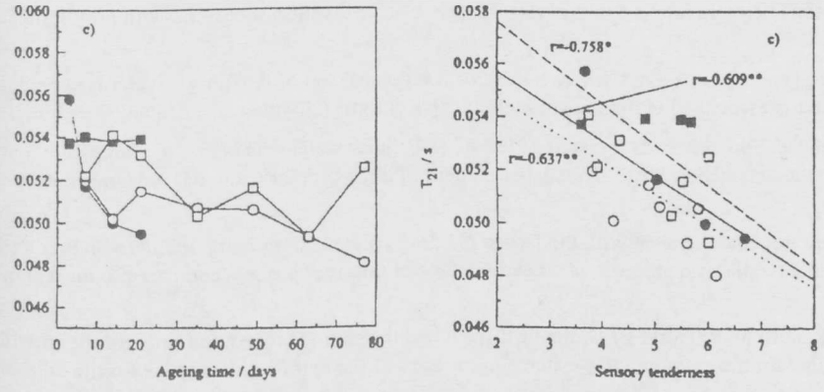

Figure 4: Transverse relaxation time, T21, of the water in the myofibrillar space as a function of ageing time and the sensory tenderness, with the storage temperature and the pH of the meat varying accordingly: normal pH aged at +4°C(●) and at –1.5°C (O) and meat with an increased pH aged at +4°C(■) and at –1.5°C (□). From Wahlgren and Tornberg, 1996.

Cooking

During cooking of meat the different meat proteins denature. This causes structural changes of the meat such as transversal and longitudinal shrinkage of meat fibers, the aggregation of sarcoplasmic proteins and the shrinkage of the connective tissue (Tornberg et.al., 1997).

The changes occurring in water proton relaxation times on cooking of meat have been studied by Fjelkner-Modig and Tornberg, 1986, Tornberg and Larsson, 1986 and Tornberg et. al., 1993a. In Figure 5 the percentage water having the slowest relaxation time T22 of 100-150 ms, using proton pulse NMR, and the extra cellular space around fiber bundles, evaluated by light microscopy, can be compared at different cooking temperatures of the meat. As has been stated before the percentage extra cellular water determined using the two methods are in relative agreement. We can moreover observe from this Figure that the amount of water around the fiber bundles increases up to 50°C in comparison with the raw meat, which seems to be in accordance with the transverse shrinkage of fibers and fiber bundles that starts to take place at temperatures of 35-40°C.

Above 50°C this widened gap diminishes again up to 70°C, probably mainly due to the shrinkage of the connective tissue starting around 60°C. It is then presumed that water is expelled by the pressure exerted by the shrinking connective tissue on the aqueous solution in the extra cellular void, causing the great water loss that is obtained on cooking. The observed increased extra cellular space from 70 to 90°C is more difficult to evaluate, but as suggested by the micrographs a swelling of the perimysium seems to occur at these temperatures, that might explain these observations.

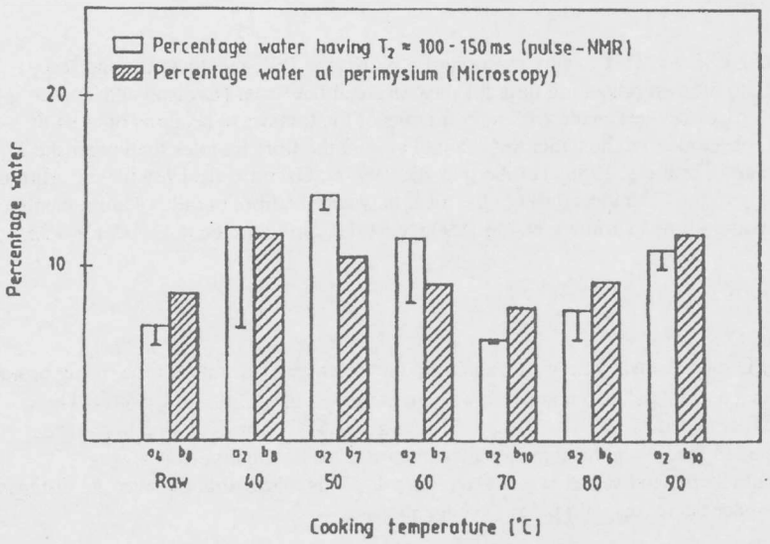

Figure 5: Percentage water having a T22 of 100-150 ms, using proton pulse NMR, and percentage water around fiber bundles (at perimysium), as determined with light microscopy, as a function of cooking temperature (a_n: n = number of samples; b_n: n = number of photos).

CONCLUSIONS

- Important quality traits of pork and beef have been considered in relation to the water distribution of the meat measured with proton pulse-NMR.
- Two dominating, discernible relaxation processes are generally observed, where the major fraction (80%) of the muscle water is considered as mainly intracellular water, whereas the remaining water is associated with the extra-cellular space, especially the water around fiber bundles.
- The water distribution in LD of purebred Hampshire and Yorkshire was registered. The extra-cellular water was higher in Hampshire than in Yorkshire, when raw, but was the other way around, when cooked to 80°C. The higher content of intra-cellular water of the Hampshire meat, when cooked, gave a more tender meat.
- During rigor development of beef LD it was demonstrated that the 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 compared to meat subjected to a slower pH drop.
- When comparing the three different qualities of pork, normal, PSE and DFD, the PSE-meat had the shortest relaxation time for the myofibrillar water and the DFD-meat had the shortest relaxation time for the extra-cellular water. This suggests least extra-cellular space for the DFD-meat.
- The relaxation time of myofibrillar water is significantly decreased by ageing time, when beef LD was stored at 4 and -1.5°C from 3 to 77 days, respectively. It was suggested that this observation reflects a disordering of the myofibrillar structure on ageing.
- When cooking beef LD from 40 to 90°C the amount of water around fibre bundles increases up to 50°C compared to raw meat, which seems to be in accordance with the transverse shrinkage of fibers and fiber bundles. Above 50°C this widened gap diminishes, again up to 70°C, probably mainly due to the shrinkage of the connective tissue.

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