Changes in water distribution of beef muscle during cooking - as measured by pulse-NMR.

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Introduction

The water-holding of meat during cooking is of the utmost importance since very substantial losses can occur (up to about 40%). From both the eating quality and economical points of view it is desirable to minimize such cooking losses.
Hamm (1972) pointed out the importance of capillary forces in holding water in meat, likening meat to a polyelectrolyte gel. Only a minor amount of the tissue water (4-5% of the total water) can be considered as bound. This water is often called hydration water and it is restricted in motion due to the proximity of the protein molecules (Wismer-Pedersen, 1971; Hamm, 1975).
From this somewhat general approach Offer et al. (1983, 1984a and b) have recently studied the water-holding of meat from a more structural point of view. This has been made possible by the use of microscopy, especially for whole meat, as it is such a highly ordered system. Offer (1984b) summarises the current state of knowledge regarding structural changes occuring on cooking, thus : Transverse shrinkage to the fibre axis occurs mainly at 40-60°C, which widens the gap already present at rigor between the fibres and their surrounding endomysium. At 60-70°C the connective tissue network and the muscle fibres co-operatively shrink longitudinally, the extent of shrinkage increasing with temperature. This longitudinal shrinkage causes the great water loss that is obtained on cooking. It is then presumed that water is expelled by the pressure exerted by the shrinking connective tissue on the aqueous solution in the extracellular void. When studying the structural causes of changes in water-holding of whole meat on cooking microscopy is a helpful tool. However, trying to quantify these structural changes by using this technique can be a tedious and laborious task. In this respect the studying of water-holding in meat using the ^{1}H-pulse-NMR (Nuclear Magnetic Resonance) technique could be more advantageous.
The multiexponential decay of the transverse relaxation time (T_2) of water protons in muscles from various sources, as measured by pulse-NMR, have been reported in the literature (Belton et al, 1972; Hazlewood et al, 1974; Pearson et al, 1974; Chang & Hazlewood, 1976; Tornberg & Nerbrink, 1984; Renou et al, 1985). Three discernible relaxation processes are mostly observed, reflecting different domains of water within the meat; thus enabling us to determine the water distribution within the meat using pulse-NMR. Lillford et al (1980) pointed out, though, that these discrete water domains do not have to arise from the structural domains seen in histological pictures of meat. The multiexponential decay of T_2 is instead explained in terms of heterogenous mass distribution down to ≈ 10 µm.
In this paper we will report on some measurements on the temperature induced changes in the water distribution of meat, as detected by pulse-NMR, which is then compared to actual water loss and to structural changes as observed by microscopy.

Materials and methods

Sample handling

M. longissimus dorsi (LD) was taken from two young bulls (electrically stimulated) four days post mortem. All samples had normal pH-values. Half the LD muscle from one bull was cut into 9 slices 1,5 cm thick, and chemical and water distribution (pulse-NMR) analysis were carried out on each slice. One third of the LD muscle from another bull was used for pulse-NMR measurements and histology preparations on raw and cooked, drained meat.

Cooking loss

Four small rods of meat for each temperature, approximately 7 mm long and 35 mm^2 in cross sectional area, were cut from the centre of the meat slice. The rods were cut carefully to avoid drip formation and put into a NMR tube (ø 7,5 mm) with the fibres prependicular to the tube wall. The weights were noted. Alle saples were thermostatically held at 25°C for 30 minutes. NMR-measurements on 3 raw samples were then taken immediately. The others were cooked in different water-baths at 40, 50, 60, 70, 80 and 90°C for 30 minutes. The samples were allowed to cool and were then thermostatically held at 25°C for another 30 minutes before NMR measurements. NMR-measurements were also carried out on cooked drained meat. The cooking loss was noted.

Water distribution by proton-pulse-NMR

The water distribution was recorded by a proton-pulse-NMR instrument (Bruker, Minispec, PC/20), mainly following the procedure of Tornberg & Nerbrink (1984). The transverse relaxation time (T_2) of the water protons within the meat was recorded at a frequency of 20 MHz at 25°C by using the Carr-Purcell-Meibom-Gill method (Meibom & Gill, 1958). The T_2-recordings were made for 2τ-spacing 4000 µs and at each measurement 64 scans were accumulated.
The magnetisation values M_0 (corresponding to 100% water protons) and M_∞(corresponding to no water protons) were calculated from the water content of the meat and by using calibration curves obtained from measurements carried out on distilled water and on an empty NMR-tube at different attenuations. $M_\infty$was used to take into account those water protons out of time window. The actual M_0 derived from the intercept of the semi-log plot of log magnetisation versus time was larger than M_0 calculated according to the description above. This was assumed to be due to cross-relaxation involving non-water protons at the protein molecules (Edzes, H.T. & Samulski, E.T., 1978; Koenig, S.H., Bryant, R.G., Hallenga, K. & Jacob, G.S., 1978). The cross relaxation was considered to be restricted only to the hydration layer of water, i.e. all relaxation processes of about 10 msec to 2 sec were supposed to derive from water protons only. In order not take into account the protons derived by cross relaxation the relaxation data were firstly analysed in multiexponential decay, for the two longest relaxation times, by curve decomposition using a microcomputer (Luxor ABC 806) as described by Tornberg and Nerbrink (1984). The fraction of water protons relaxing according to the two longest relaxation times was substracted from M_0 (100% water protons) together with those water protons out of the window. Residual water

protons thus obtained were taken as those relaxing the fastest. A magnetisation value was read off somewhere between 15 and 45 ms from the FID-curve and was put into the equation describing multiphasic relaxation given by Zimmerman and Brittin (1957). The relaxation time of the fastest relaxing water protons was then calculated using the equation.

Water distribution by microscopy

Rods of approximately 3,5 g (two for each temperature) were cut from the centre of the meat slice and put into test tubes with the fibres parallel to the tube walls. The samples were cooked for 30 minutes in different water baths held at 40, 50, 60, 70, 80 and 90°C.
The piece of cooked meat was cut into two halves. One was used for histological preparation and the other for NMR measurements and water contentanalysis. Histological preparation and NMR-measurements were also made on raw meat.
The meat samples were fixed overnight in 4% glutaraldehyde and then washed in phosphate buffered saline for 1 hour. Embedding was made with 5% agar according to Kotter (1955) and the embedded samples were then frozen and mounted with the muscle fibres perpendicular to the knife in a cryostat microtome (type TE, SLEE, London). The microphotographs were taken with a Nikon Optiphot light microscope. The negatives were mounted in an enlarging apparatus and the outlines of the frame and the extracellular space around perimysium was drawn by hand on a paper at a total magnification of 300X. The total area and the area of extracellular space around the fibre bundles were cut and weighed. By assuming even distribution of water all over the meat sample the percentage water at the extracellular space around the fibre bundles (at the perimysium) was calculated.

Results and discussion

In figure 1 the percentage protons of the discernible T_2-relaxation processes of water protons in raw, cooked and drained meat can be seen in the form of histograms. Three relaxation processes are obtained in all samples. The percentage of water having the longest relaxation time, i.e. $T_2 > 1$ s, was considered as more or less 'free water'. The relaxation time of water without any proteins solved or dispersed is about 2,5 s. By assuming that the fast exchange between small bound fraction of water adjacent to the biopolymers and the larger 'free' fraction of water is the cause of the reduced relaxation time of water protons, a long relaxation time will suggest a long diffusion distance of the 'free' water protons to the exchange site. This means that larger pores of water within the structure have a greater chance of obtaining relaxation times in the proximity to that of free water than smaller pores. Assuming that water in large pores is most likely to be drained off from the meat (capillary forces) we have compared, at every cooking temperature studied, the percentage of water having the longest relaxation time ($T_2 > 1$s) in cooked, undrained samples and the water in the cooking loss. The results of this comparison expressed as a histogram can be seen in figure 2.

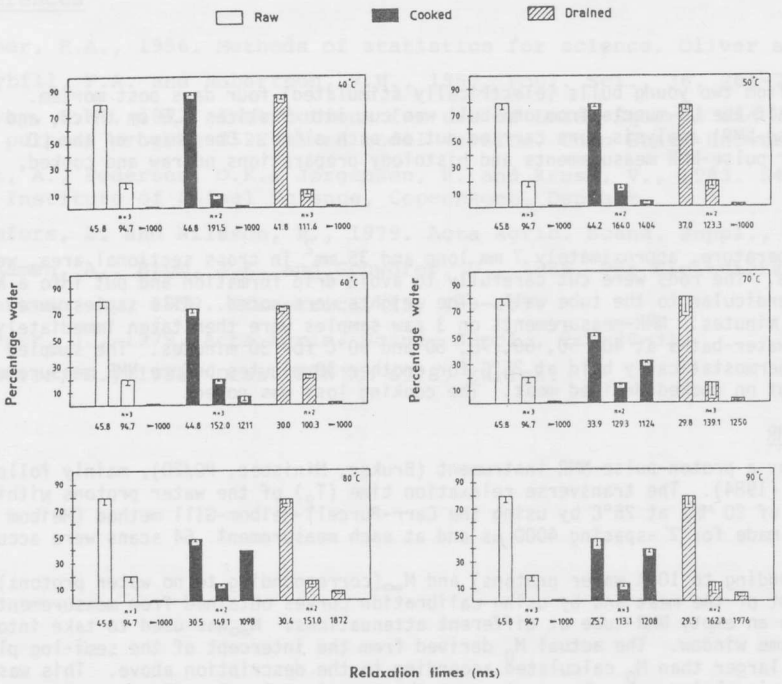


Figure 1. The percentage of water protons having discernible relaxation times for samples of whole meat (beef longissimus dorsi being raw (□), cooked (■) and cooked, drained (▨). The cooking temperature was varied from 40 to 90°C (n = number of samples).

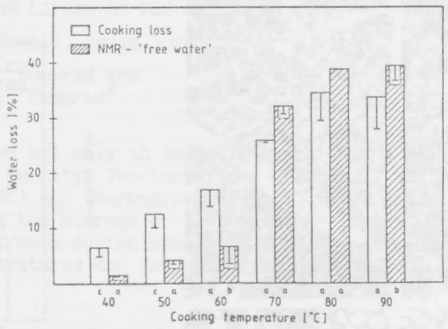


Figure 2. Water loss (%) expressed as cooking loss (□) or NMR-'free water' (▨) for meat samples of beef longissimus dorsi as a function of cooking temperature (a: n = 2; b: n = 3; c: n = 4).

For temperatures below 60°C the percentage of water drained from the sample was higher than the amount of 'free water', detected by pulse-NMR, whereas the reverse was found for temperatures above 60°C. However, the same tendency as a function of temperature is observed for both ways of studying the water holding of meat, i.e. relatively low amount of water loss for temperatures up to 60°C, at 70°C there is the largest increase in the release of water, and at both 80 and 90°C cooking will result in the highest and the same level of water loss.

We observed, when the piece of cooked meat was drawn out of the NMR-tube for weighing, that below and equal to 60°C the meat sample was more susceptible to disintegration than above 60°C. According to the microphotographs of the transverse sections of M. longissimus dorsi seen in figure 3 there is a more loose meat structure at cooking temperatures below and equal to 60°C than above this temperature. This more open structure at 60°C and below orginates mainly from larger extracellular space especially around the fibres, which was also the experience of Offer et al. (1984a) for beef psoas muscle.

Fractures will more easily occur in pieces of meat that consist of more cracks. Therefore meat cooked at 60°C and below is more susceptible to fall apart and thereby leak more water than it should. We suggest that this is the cause for cooking loss being larger than the amount of water liable for loss, as measured by pulse-NMR. At cooking temperatures at 70°C and above, however, the meat structure is more compact and those larger pores of extracellular water (now mainly at the perimysium), which are liable for loss, will be held by the compact surrounding structure. Therefore leakage of water will be prevented to a certain degree. This could be the reason for the higher amount of NMR-free water than actual cooking loss obtained at cooking temperatures of 70°C and above. This is further substantiated by the fact that the amount of 'free water' in the cooked and drained samples, as can be seen in Figure 1, is larger for the samples cooked to temperatures of 70°C and higher than those samples cooked at lower temperatures.

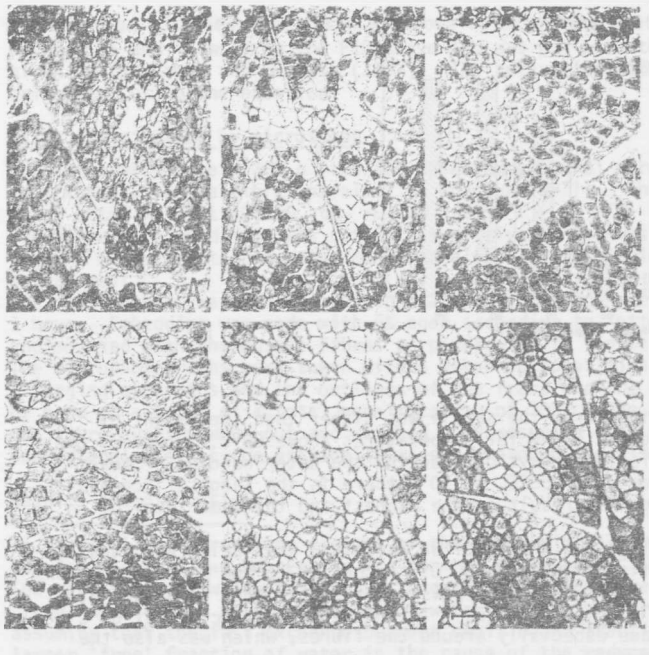
Thus, by measuring the amount of water relaxing with a relaxation time T_2 above 1 s with H'-pulse-NMR, water liable for loss is measured. The actual amount of water that is lost thus depends on the structure and the mechanical handling of the meat.

As seen in figure 1 we can for all samples see the existence of three discernible T_2-relaxation processes. The percentage of water relaxing with the shortest relaxation time (a T_2 ranging from 46 to 26 ms) can be considered as mainly holding the interfilamental and intracellular water. This reasoning is based on the following facts. A very high fraction of the muscle volume is occupied by the myofibrils in the raw material, which is in accordance with the high percentage of water of about 80% having the shortest relaxation time. Moreover, the domain of water with the shortest relaxation time has the highest protein concentration which is expected to be the space within the myofibrils.

It has been suggested from 'H-pulse-NMR measurements (Hazlewood et al, 1974, Lillford et al, 1980, Tornberg & Nerbrink, 1984) that the medium-rate relaxing process, i.e. the one with a T_2 100 -150 ms, is associated with the extracellular space. As can be envisaged from figure 3 the extracellular space around the perimysium and endomysium can, for example, for the 50°C sample constitute up to 28% (calculated by a cutting and weighing procedure). This figure clearly exceeds the value of 14% water relaxing with a T_2 around 150 ms (see figure 4). Evidently, this way of looking at the medium-rate relaxing process does not always hold true. We will instead suggest that the percentage of water-protons relaxing at the medium-rate of T_2 100-150 ms is mainly associated with the extracellular water situated around the fibre bundles. This reasoning is based on the following. The probality of a water proton exchanging with any proton of hydration water is dependent on the concentration of hydration protons within the diffusion distance of each water molecule and thus on the interfacial surface of proteins, which is highest within the myofibrils. Consequently, those water protons being furthest away from the myofibrils will have the highest probability of having a slow relaxation, i.e. those water protons situated in the middle of the extracellular space around the fibre bundles.

We have therefore compared the percentage of water having a relaxation time of T_2 100-150 ms (measured by pulse-NMR) and the percentage of water associated with the perimysium, by cutting and weighing enlarged photographs of the transverse section of fibres as seen in figure 3. The result of this comparison can be seen in figure 4 for the raw meat and for the meat cooked at the different temperatures studied.

If we compare the difference in percentage water around fibre bundles as determined by pulse-NMR and microscopy at every temperature it is on average $\pm$ 1,8%. The standard deviation in determination of percentage water by pulse-NMR is, when averaged over all temperatures, equal to $\pm$ 2,1%. This means that the error in determining the percentage water at the perimysium by pulse-NMR (20%) is of the same magnitude as the difference observed between the two methods of measurement. Evidently, the percentage of water around fibre bundles can in this study, be measured with 80% probability by the pulse-NMR method. This is probably not a general phenomenon as it depends on the actual pore size distribution of water within the meat. Therefore, in order to be able to localize the different domains of water, as measured by pukse-NMR, with some accuracy, microscopial observation of the actual meat structure is needed.



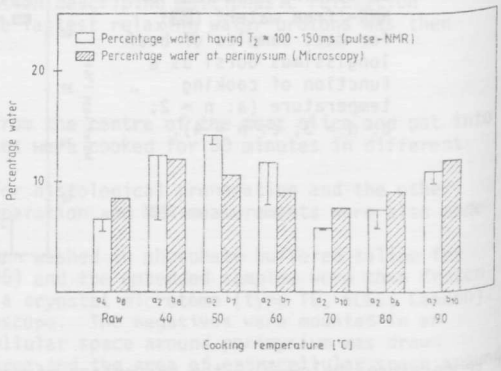


Figure 4 : Percentage water having T_2 100-150 ms (pulse-NMR,) and percentage water around fibre bundles (at the perimysium,) as determined by microscopy, as a function of cooking temperature (a_n : n = number of samples; b_n : n = number of photos.

Figure 3 : Transverse sections of beef longissimus dorsi raw (A) heated at 40°C (B), 50°C (C), 60°C (D), 70°C (E), 90°C (F). : 10 um.

In conclusion, the combined use of microscopy and proton-pulse-NMR seems a promising way of studying both quantitatively, the changes in water distribution in meat during cooking.

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