## 3:10 Swelling of whole meat and myofibrils - as measured by pulse-NMR

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### Introduction

Water-holding of meat is a major area of concern to the meat industry and to the meat research workers. A high water-holding of meat is not only favourable for economic reasons, but also sensory attributes such as juiciness and tenderness of meat are very much influenced by the water-holding capacity. A critical discussion of the literature on this subject has recently been presented by Offer and Trinick (1). By studying isolated myofibrils in different media under the microscope they arrived at the conclusion that the shrinking and swelling of myofibrils are the main causes for changes in the water-holding of whole meat.

Common methods for measuring water-holding of meat and meat products are based on the application of an external force such as pressure, centrifugation, capillary suction or temperature (cooking loss) (2). We here report on some representative investigations on the swelling of whole meat and myofibrils, by which the applicability of proton-pulse-NMR (Nuclear Magnetic Resonance) measurements with regard to water-holding of meat is shown. With this method no external force, which can cause damage to the meat structure, has to be applied. This gives the pulse-NMR method a more absolute character than former methods used. Moreover, the changes in water distribution within the meat caused by all types of meat processing can be more easily localised and quantified.

Multiexponential decay of the transverse relaxation time ($T_2$) of protons in muscles from various sources as measured by pulse-NMR have been reported (for review see Lillford et al. (3)). The elucidation of these results has given rise to opposing theories for the behaviour of water in muscle tissue. This problem have been sorted by Lillford et al. (3), who have shown experimentally that multiexponential water proton relaxation can occur in a variety of food systems such as soya protein fibres and raw and cooked muscle tissue. By assuming that fast exchange occurs betweeen a small bound fraction of water adjacent to the biopolymers and the larger "free" fraction, a theory was developed. In this theory the experimentally observed multiexponential decay of $T_2$ can be explained in terms of heterogenous mass distribution down to ≈10 μm. This theory is also the basis for elucidating the water proton relaxation behaviour of myofibrils and whole meat observed in this study.

### Materials and Methods

Muscle tissue from M. longissimus dorsi (2 young bulls) was taken 2-7 days post-mortem. The samples of meat were taken from the middle part of the muscle by cutting a transverse mid section. pH was measured at 4-6 places along this muscle section. Ultimate pH for muscle 1 was 5.6 and for muscle 2 5.4. The water and fat content of the two muscles was 75% and 1.5%, respectively. Approximately 2.5 g of whole tissue from muscle 1 was placed in a NMR tube (Ø 13 mm) and thermostatted at 40°C for 30 minutes before the pulse-NMR measurements. For swelling studies whole meat samples of the same size were placed in an E-flask with isotonic buffer [100 mM KCl, 20 mM K-phosphate (pH = 7.0), 1 mM EDTA and 1 mM $NaN_3$] or 0.8 M NaCl solution at 40°C for 30 minutes. The piece of meat was then dried of excess water and put in the NMR-tube for direct measurement.

Myofibrils from muscle 1 and 2 were isolated according to the procedure given by Olson et al. (4) for the determination of myofibrillar fragmentation index. The mixer used in the procedure was an Omni-mixer used at 11 000 rpm for 60 seconds. After the last centrifugation (2°C) the sediment of the myofibrils was transferred to a NMR tube (Ø 13 mm, 2.5 g and 40°C or Ø 7.5 mm, 0.5 g and 25°C) and thermostatted for 30 minutes before measurement. For swelling studies the sediment of myofibrils was resuspended in 20 ml isotonic buffer, 0.4 M NaCl or 0.8 M NaCl solution in a whirlmixer for 10 seconds. The suspension of myofibrils was then directly centrifuged (2°C) at 1000 g for 15 minutes. The sediment was transferred to a NMR-tube and thermostatted at the measurement temperature for 15 minutes before being measured.

The measurement of the transverse relaxation time ($T_2$) of the water protons within the meat and the myofibrils was made using a BRUKER PC 20 pulse spectrometer at a frequency of 20 MHz. $T_2$-measurements were made by using the Carr-Purcell-Meibom-Gill method (5). τ-spacings for all the samples were set to 4000 μsec. Magnetisation decay could with this τ-spacing be registered from 8 ms up to 1344 ms. The last measuring time is then in the vicinity of the "free" water proton relaxation time (≈2 seconds), where most of the water protons have relaxed. With this large τ-spacing used, a large time-window is covered but the accuracy of determining the $T_2$ of the fastest relaxing protons is reduced. 100 scans were accumulated in each case.

Measurements of muscle 1 were performed at 40°C and muscle 2 at 25°C. The relaxation data were analyzed in multiexponential decay by curve decomposition with a microcomputer (Luxor, ABC 80). We followed the usual procedure of curve decomposition by finding the slowest decaying fraction by fitting the data in a semilog plot of log magnetisation versus time to a straight line by linear regression analysis. The data from this line were subtracted from the observed data and a new semilog plot was formed. This plot was then decomposed as described above until no further straight lines could be fitted to the data. $T_2$ for every exponential decay was calculated by the inversion of the slope of each straight line obtained. The relative population of protons relaxing according to the different $T_2$ found was derived from the intercept of each straight line at time 2τ (= 8 ms) in the plot of log magnetisation versus time. The magnetisation at time 2τ was set to correspond to 100% protons. The criteria for best fit was the highest correlation coefficient possible for all the observed discernible exponential decays of $T_2$. This was achieved by an iterative procedure. The error introduced by this calculation procedure is estimated on average to be for the slowest relaxing process ±20-40 ms, for the next process ±2-4 ms and for the fastest ±1-2 ms. The accuracy of the determination of the relative population of protons in each process is for the slowest process ±0.1%, for the next process ±1-2% and for the fastest process 3-4%.

### Results and Discussion

In table 1 the discernible $T_2$-relaxation processes of water protons in the different whole meat samples are tabulated. Three relaxation processes are observed in all cases. The relaxation of protons is dominated (75-80%) by a $T_2$-relaxation of ≈35 ms. As is emphasised by Offer et al. (6) a very high fraction (80-87%) of the fibre volume is occupied by myofibrils, which suggests that the interfilamental water (myoplasm) has a $T_2$-relaxation of about 35 ms at the temperature of 40°C. Hazlewood et al. (7) found a transverse relaxation time of 45 ms for 82% of the tissue water (rat: M. gastrocnemius), which is similar to the 47 ms to 90% content in M. longissimus dorsi (heifer) of Lillford et al. (3). Those authors, however, have a measuring temperature of 25°C and 14°C, respectively, which can give rise to the observed differences in $T_2$. We have also made a measurement of isolated myofibrils at a lower temperature of 25°C, the results which are shown in Table 3. The fastest relaxation time of 48.6 ms shown up in these experiments is also more in accordance with those relaxation times reported in the literature (3, 7).

Hazlewood et al. (7) found 10% of the tissue water to be associated with extracellular space and the relaxation time to be four times that of the myoplasm. This seems to be in accordance with our observed 120 ms-$T_2$-relaxation of a relative population of about 13%. Offer et al. (6) speculate that when muscle enters the rigor state the myofibrillar volume diminishes by 10 to 20%. This is the same sort of magnitude observed in this study.

For the "unswollen" meat samples only about 90% of the water protons are detected. When such large τ-spacings as 4000 μs are used the rest of the water (≈10%) probably relaxes too quickly to be detected. Hazlewood et al. (7) found 8% of the water to be associated with the macromolecules in the tissue, these protons relaxing in a shorter time than 8 ms (=2τ).

For those whole meat samples equilibrated with isotonic buffer or 0.8 M NaCl solution for 30 minutes no striking changes in swelling can be observed. The amount of detectable water does however increase for the "swollen" meat pieces, and there is a tendency for a higher amount of extracellular water in those samples.

It should be noted in connection with interpreting the results that $T_2$-relaxation of isotonic buffer, 0,4 M NaCl and 0.8 M NaCl solution, has been measured to vary between 2.5-3.0 s, which is more or less the same as for free water. This observation enables us to assume that the shift in $T_2$ is mainly due to structural rearrangements as, for example, swelling and shrinking of myofibrils.

| Sample | $T_2$ (ms) | Relative population of protons (%) |
|---|---|---|
| Not equilibrated (Prep. 1) | 613 | 1.2 |
| | 114 | 12.6 |
| | 36.3 | 75.9 |
| | | 89.7 |
| Not equilibrated (Prep. 2) | 881 | 1.5 |
| | 126 | 13.7 |
| | 34.3 | 76.1 |
| | | 91.3 |
| Equilibrated in isolating buffer | 674 | 3.5 |
| | 124 | 14.0 |
| | 37.1 | 80.2 |
| | | 97.7 |
| Equilibrated in 0.8 M NaCl-solution | 788 | 1.2 |
| | 104 | 15.4 |
| | 37.4 | 76.6 |
| | | 93.2 |

Table 1. $T_2$-proton relaxation of whole meat (muscle 1) measured at 40°C 2 days post-mortem not equilibrated and equilibrated in isotonic buffer and in 0.8 M NaCl-solution for 30 minutes.

When myofibrils are isolated from whole muscle tissue a substantial swelling of the myofibrils occurs, clearly shown in the results of Table 2. Most of the protons (75-80%) are now

relaxing according to a $T_2$ of 110-120 ms. This observation is important in two respects. Firstly, it indicates that most of the water in meat is held within the myofibrils, thus agreeing with the observations made by Offer et al. (1,6). Secondly, the substantial swelling of myofibrils when extracted from muscle tissue points to the important role that the sarcolemma and the endomysium have in inhibiting swelling of the myofibrils. Matsubara and Elliot (8) and Magid and Reedy (9) have also observed that when fibers are skinned they swell substantially.

| | Sample | $T_2$ (ms) | Relative population of protons (%) |
|---|---|---|---|
| 5 days post mortem | Myofibrils in isolating buffer | 1465<br>119<br>36.6 | 3.9<br>79.5<br>8.6<br>92.0 |
| | Myofibrils in 0.4 M NaCl | 125.9 | 93.7 |
| | Myofibrils in 0.8 M NaCl | 452<br>154 | 71.6<br>32.7<br>104.3 |
| 7 days post mortem | Myofibrils in isolating buffer | 1808<br>113 | 1.4<br>74.3<br>74.7 |
| | Myofibrils in isolating buffer | 2190<br>112 | 1.8<br>77.1<br>78.9 |
| | Myofibrils in 0.4 M NaCl | 162<br>94.6 | 58.5<br>41.3<br>99.8 |
| | Myofibrils in 0.8 M NaCl | 363<br>155 | 48.7<br>51.4<br>100.1 |

Table 2. $T_2$-proton relaxation of isolated myofibrils (muscle 1) measured at 40°C 5 and 7 days post-mortem in isotonic buffer, 0.4 M NaCl and 0.8 M NaCl-solution.

In some preparations of the myofibrils, as for example for those made 5 days post mortem, the 35 ms-relaxation can still be recorded. We interpreted this as 9% of the myofibrils still being covered by the endomysium and the sarcolemma, and they are therefore not swollen. In some recent investigations (10) on the collagen content of myofibrils after isolation as much as 20% of the original content of collagen in the muscle could be left in the preparation. It therefore seems plausible that at least half of it exists as endomysium sheets surrounding some of the myofibrils and thereby inhibiting their swelling.

As can be seen from the results presented in Table 2, further noticeable swelling occurs when the myofibrils are resuspended in salt solutions, particularly in 0.8 M NaCl solution. This was not the case for whole meat when equilibrated in 0.8 M NaCl solution for a similar length of time. Evidently, for substantial swelling of the myofibrils to occur through salt addition, the myofibrils should have been laid bare.

It is interesting to compare the swelling of myofibrils when resuspended in 0.4 M NaCl and 0.8 M NaCl solution. In the former case the $T_2$-relaxation of 110-120 ms is still the dominating relaxation process, but now to a higher relative population. This probably means that the structural arrangements of the myofibrils have not changed much compared to those in isotonic buffer, but there is a larger number of myofibrils at this stage. However, when myofibrils are put in 0.8 M NaCl solution the $T_2$-relaxation of 110-120 ms vanishes and is substituted by two $T_2$-relaxation processes of 150-160 ms and 350-650 ms. Evidently, the myofibrils have swollen dramatically and the myofibril preparation has now a more gel-like appearance. Offer et al. (1, 6) have shown by microscopical evaluation of myofibrils that no dramatic swelling occurs until 0.6 M NaCl solution is used, which agrees with the observations made in this study.

It is also interesting to compare the swelling of myofibrils when extracted at different times post-mortem; although one should bear in mind that variations can occur from one preparation of myofibrils to another. One of the main causes to this variation is suggested by recent investigations (11) to be due to meat quality differences within the muscle. The results presented in Table 2 still give an indication of what can happen during late post mortem storage.

For the myofibrils extracted 7 days post mortem only 75-80% of the water protons are recorded in the time window investigated. Of the 20-25% fast relaxing water protons some could be associated with an increased amount of denatured and aggregated myofibrillar proteins. This increased denaturation of myofibrillar proteins could be induced by the high measuring temperature of 40°C, as temperatures between 35°C and 42°C have been shown to cause myosin denaturation, particularly at pH-values around 5.5 (12). Nevertheless, the susceptibility of the myofibrillar proteins to denature seems to increase as a function of post-mortem storage.

When the myofibrils extracted 7 days post mortem are swollen by salt addition 100% of the water protons are registered again, but the swelling pattern is not exactly the same as for those myofibrils extracted 5 days post mortem. In the 7 days post-mortem sample and in 0.4 M NaCl the aggregated proteins probably contribute to the faster 95 ms-relaxation process compared to the 120 ms-relaxation observed for the 5 days sample. For the 7 days sample in 0.8 M NaCl the same relaxation times turn up again as for the 5 days sample, but the relative populations have changed.

We have also measured isolated myofibrils from the same muscle from another animal 3 days post-mortem. The results can be seen in Table 3. In this case the measuring temperature was lowered to 25°C. As we know that myosin is susceptible to denaturation at 40°C (12), we wanted to check if the observations made were induced only by the high measuring temperature.

According to Table 3 these myofibrils in isotonic buffer have 75% of the water protons relaxing with a $T_2$ of ≈120 ms, which is similar to the results given in Table 2. (There is a small shift towards a slower relaxation, which is most probably due to the lower measuring temperature.) But in this preparation almost 20% of the myofibrils seem not to have been squeezed out of the endomysium sheets. This is a figure which agrees with our observations on the collagen content of the isolated myofibrils (10).

| Sample | $T_2$(ms) | Relative population of protons (%) |
|---|---|---|
| Myofibrils in isolating buffer | 5363<br>124<br>48.6 | 2.2<br>75.6<br>20.2<br>98.0 |
| Myofibrils in 0.4 M NaCl | 821<br>110 | 4.1<br>90.6<br>94.7 |
| Myofibrils in 0.8 M NaCl | 650<br>164 | 11.7<br>88.3<br>100.0 |

Table 3. $T_2$-proton relaxation of isolated myofibrils (muscle 2) measured at 25°C 3 days post-mortem in isotonic buffer, 0.4 M NaCl and 0.8 M NaCl-solution.

As for the 5 days stored muscle 1 the addition of 0.4 M NaCl only increases the number of protons relaxing in the $T_2$-region of 110-120 ms, but it does not alter the relaxation time. The 150-160 ms-relaxation time (somewhat slower due to the lower measuring temperature) turns up again, when the myofibrils are dispersed in 0.8 M NaCl, but in this case for about 90% of the protons.

## Conclusion

This report shows the applicability of proton pulse-NMR for investigations on the swelling of whole meat and myofibrils. Further investigations on the phenomena observed in this study, using this new technique, are now in progress in our laboratory.

## References


1. Offer, G. and Trinick, J. Meat Science 8, 245 (1983).
2. Hamm, R. Die Fleischerei 9, 590 (1982).
3. Lillford, P.J., Clark, A.H. and Jones, D.V. From ACS Symposium Series No. 127, 177 (1980).
4. Olson, D.G., Parrish, F.C. and Stromer, M.H. J. Food Sci. 41, 1036 (1976).
5. Meiboom, S. and Gill, D., Rev. Sci. Instrum. 29, 688 (1958).
6. Offer, G., Restall, D. and Trinick, J. In Recent Advances in the Chemistry of Meat, p. 71, April (1983).
7. Hazlewood, C.F., Chang, D.C., Nichols, B.L., Woessner, D.E. Biophys. J, 14, 583 (1974).
8. Matsubara, I. and Elliott, G.F. J. Mol. Biol. 72, 657 (1972).
9. Magid, A. and Reedy, M.K. Biophys. J. 30, 27 (1980).
10. Tornberg, E. and Fjelkner-Modig, S. To be published.
11. Tornberg, E. and Nerbrink, O. To be published.
12. Yasui, T., Gotoh, T. and Morita, J. J. Agric. Food Chem. 21, 241 (1973).