S4P23.WP

WATER DISTRIBUTION IN RAW PORK MUSCLE (M.LONGISSIMUS DORSI) OF DIFFERENT MEAT QUALITIES

E. TORNBERG, A. ANDERSSON AND G. VON SETH

Swedish Meat Research Institute, POB 504, S-244 24 Kävlinge, Sweden

Please refer to Folio 34.

INTRODUCTION

Water losses from whole raw meat, drip loss, is obtained when a muscle is cut. This fluid, a solution of sarcoplasmic proteins, is drained from the cut surface of the meat by gravity, if the viscosity of the water is low enough and the capillary forces do not retain it. Offer *et al.* (1989) have confirmed that the drip arises predominantly from the longitudinal channels through the meat between the fibre bundles. The main question then arising, in order to be able to control and understand changes in drip loss, is how water is accumulated in those channels.

Most of the water in the living muscle is held within the myofibrils (80%), in the spaces between the thick and thin filaments (Offer *et al.*, 1989). Any larger changes in the distribution of water within the meat structure that occurs during the rigor period post-mortem, then, by necessity, originate from changes in this spacing. Lateral shrinkage of the filament lattice can be brought about by a pH-fall closer to the isoelectric point , rigor contraction and myosin denaturation (Offer and Knight, 1988). There will only be changes in the water distribution in the myofibrils then change in volume. This will only occur in the two latter cases, whereas electrostatic shrinkage induced by pH-changes will cause longer sarcomere length, i.e. the myofibrillar volume is constant. The fact that fibre and fibre bundles shrink when their constituent myofibrils shrink, has been shown by Offer and Cousins (1992), thereby giving rise to the two extracellular compartments around fibres and fibre bundles. Another origin of changes in the water distribution within meat, rarely discussed in the literature, is the differences in osmotic pressure across the cell membrane that can cause swelling and shrinkage of the fibre.

A powerful tool for studying the distribution of water in the muscle is the non-invasive ^{1}H-pulse-NMR (Nuclear Magnetic Resonance) technique. Most of the water in raw muscle is free and only a minor amount (4 to 5% of the total) can be considered as bound. This water is often called hydration water and is restricted in motion due to the proximity of the protein molecules (Hamm, 1975). Water protons in meat have shorter transverse relaxation times (T_2) than those in bulk water. This is explained by a fast exchange between free water and the hydration water adjacent to the proteins. A long relaxation time suggests 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 of that of free water than water in small pores. Therefore, the water distribution in pores of different sizes can be studied using proton pulse-NMR.

The multiexponential decay of the T_2 of water protons in pork muscle has been reported on in the literature (Renou *et al.*, 1985; Fjelkner-Modig and Tornberg, 1986; Larsson and Tornberg, 1988; Renou *et al.*, 1989; Borisova and Oreshkin, 1992; Tornberg *et al.*, 1992). Two dominating, discernible relaxation processes have mostly been observed, where the major fraction (80%) of the muscle water has a T_2 between 35-50ms, while the rest of the water relaxes in the range of 100-150ms. The percentage of water relaxing with the shortest relaxation time can be considered as mainly hold by the myofibrils, since a very high fraction of the water is occupied by the myofibrils. Lillford *et al.* (1980) pointed out, however, that these discrete water domains do not necessarily have to arise from the structural domains seen in the histological pictures of meat. The multiexponential decay of T_2 is generally explained in terms of heterogeneous mass

distribution down to 10m. Tornberg and Larsson (1986) have shown, by comparing the percentage of water having a T_2=100-150ms with the percentage of area around the fibre bundles (evaluated by microscopy), that the latter could be predicted with an 80 % probability using the pulse-NMR method.

Abberations in the quality of pig meat, such as pale, soft and exudative meat (PSE) and dark, firm and dry meat (DFD), have been registered using ^{1}H-pulse-NMR-measurements (Renou *et al.*, 1985; 1989; Larsson and Tornberg, 1988; Tornberg *et al.*, 1992). If the meat is pale and the drip loss is high, it is usually called PSE meat, whereas dark meat of enhanced ultimate pH (>6.0 in LD) is called DFD meat.

Lopez-Bote *et al.* (1989) have studied the extent to which myofibrillar and sarcoplasmic proteins are denatured in PSE, normal and DFD meat. They found that the amount of denatured sarcoplasmic proteins was significantly different from normal in both PSE and DFD muscles, whereas both sarcoplasmic and myofibrillar proteins were denatured in PSE samples. Literature on the meat structural changes of PSE meat is relatively scarce. With regard to longitudinal contraction, Honikel and Kim (1986) showed that for muscles with pH_{45}<5.8 they had longer sarcomeres than in normal meat. With regard to lateral shrinkage Offer and Knight (1988) have shown with X-ray diffraction studies on PSE muscle at 24 hours post-mortem, that the post-slaughter shrinkage of myofibrils is about twice that of normal pigs. A larger extracellular volume has also been observed with light microscopy for PSE meat, compared to normal (Penny, 1977; Larsson and Tornberg, 1988). Swatland (1988) found that the myofilament distance varied from 39 to 49nm between weak PSE and weak DFD.

Parts of the data in this article have already been published elsewhere (Larsson and Tornberg, 1988; Tornberg *et al.*, 1992). In the latter article the meat quality and structural traits of PSE, normal and DFD meat have been related to the sensory properties of cooked meat, whereas in the former an overall view of the structural traits of different qualities of pork meat was obtained. We would, in this paper, like to penetrate this data further, focusing more on the mechanisms responsible for differing water-holding in PSE, normal and DFD meat.

MATERIALS AND METHODS

M.longissimus dorsi (LD) was taken from 40 pigs of differing meat quality, i.e., normal (N), PSE and DFD as described by Larsson and Tornberg (1988).

Chemical analysis

The water (Nilsson, 1969) and fat (SBR-method, NMKL, 1974) contents were determined in a 4cm section of the LD muscle removed at the last rib.

Meat quality traits

The following meat quality parameters were measured: ultimate pH, internal light reflectance (FOP-values) and drip loss as described by Larsson and Tornberg (1988). Pig meat with FOP-values ≥55 was considered to be PSE and pigs with ultimate pH≥6.0 to be DFD.

Meat structure traits

Proton-pulse-NMR

The water distribution was recorded as described by Larsson and Tornberg (1988) and the relaxation data was analyzed as described by Tornberg and Nerbrink (1984).

Microscopy

Microphotos of cross-sectional cuts from LD were taken according to the description by Larsson and Tornberg (1988). Using an image analyzing system, LAB EYE 3.07 (Innovativ Vision AB, Sweden), the distance, x, between fibre bundles was determined as described by Tornberg *et al.* (1992).

Sarcomere length

The lengths of the sarcomere diffraction bands were recorded using a helium-neon laser as described by Larsson and Tornberg (1988).

Statistical analysis

Data was analyzed statistically with the SYSTAT programme (SYSTAT, 1987) using t-test and linear regression analysis.

RESULTS AND DISCUSSION

The mean values of the measured meat quality and the structural traits of the raw meat are gathered in Table 1, for the three different quality groups normal (N), PSE and DFD. Drip loss differed significantly between the groups, whereas normal and PSE meat had a similar ultimate pH. There is no significant difference in sarcomere length between the different quality groups, but the variation in sarcomere length is largest in the PSE group and smallest in the DFD group.

A three-component T_2-relaxation behaviour was mostly observed in the ^{1}H-pulse-NMR-measurements, indicating that there are three water regions that can be quantified. The longest relaxation time was regarded to be the "expelled water" and was excluded from the results (not exceeding 1 % of the water). The major fraction (70-94%) of the muscle water had a relaxation time (T_{23}) between 35-50ms. The rest of the water relaxed in the range of 70-180ms (T_{22}).

According to Table 1, the DFD group had the fastest T_{22}, while the PSE group tended to have the slowest. As observed earlier (Larsson and Tornberg, 1988), the meat quality parameters characterising the raw meat, i.e., the drip loss, the FOP-values and the ultimate pH, were best correlated with the T_{22}-relaxation time, out of all the data obtained from the pulse-NMR-measurements (drip: r=0.60***, FOP: r=0.61***, pH: r=-0.67***). Evidently, the T_{22}-relaxation time reflects a meat structural property of importance to meat quality.

As reasoned in the introduction, the T_2-relaxation time reflects the diffusion distance of a proton to the exchange site of the hydration water. We have chosen three samples from each quality group (N, PSE and DFD). For these samples the average distance between fibre bundles, x, was evaluated from the micrographs of cross-sectional cuts using an image analyzing system. In some cases (PSE meat), the gaps between fibres within a fibre bundle were large. For those samples, the gaps between fibres were included in the measurement. Assuming that the proton migration to the exchange site is diffusion-controlled, the average time, t, for the molecule to travel the distance, x, when the diffusion coefficient is D, is given by Einstein, 1956 in accordance with the formula:

$$t = \frac{x^2}{2D}$$

The average time, t, should in fact be related to the T_{22}-relaxation time registered in the pulse-NMR-measurements. Thus, we have plotted the T_{22}-relaxation time for the nine samples in Figure 1 as a function of the squared distance, x^2. According to the formula, this should give a straight line, which is obtained in Figure 1 with a correlation coefficient of r=0.85***. Our results indicate the importance of the size of the water compartment outside the fibre bundles for the water-holding of raw meat, in accordance with Offer *et al.* (1989).

For further evaluation of the results, we have analyzed the three meat quality groups separately.

As seen in Table 1, the drip loss for the six DFD samples obtained is small and varies little (s.d.=0.3). Therefore the volume of the myofibrillar space can be considered to be more or less constant within this group. However, the sarcomere length increases significantly (r=-0.80*) with the lowering of pH during rigor (Figure 2), which suggests there is a restricted lateral shrinkage of the myofilament lattice, due to the high ultimate pH. Further evidence for the lateral shrinkage of myofibrils upon lowered pH in DFD meat from 6.4 to 6.0 is given in Figure 3. In this figure the light scattering of the meat (FOP-value) is positively, linearly related to the sarcomere length (r=0.81*). By lowering the pH, the sarcomere length is increased (Figure 2) and consequently the myofibres shrink laterally. Offer and Knight (1988) argue that myofibrils themselves are the major source of light scattering in meat. This can especially be true for DFD meat, where the denaturation of proteins is at a minimum. From the same reference it is referred that Jeacocke

(unpublished results) has shown that light scattering is at a maximum, when the myofibrillar diameter is at a minimum. This reasoning fits the results presented in Figure 3, where light scattering increases (albeit from a low value), when the myofibrillar diameter declines.

For PSE meat, drip loss varied from 2 to about 6.5% (Figure 4) and was best linearly correlated to the T_{23}-relaxation (r=-0.77*). That is in accordance with Offer *et al.* (1989), who stated that the denaturation of myosin heads occurring in PSE meat leads to the lateral shrinkage of myofibrils. This in turn gives rise to the shrinkage of the myofibrillar space, causing a higher protein concentration in the myofibrillar volume and consequently a lower T_{23}-relaxation time. This T_{23}-dependence was not observed in the DFD meat, although there was a variation in the lateral shrinkage of the myofibrillar space. But as the myofibrillar volume was more or less constant for this type of meat, protein concentration did not vary and no T_{23}-differences were observed.

For normal meat there is a substantial variation in drip loss from 1 to 5% (Figure 5). This drip loss is mainly governed by pH (r=-0.58**), but not by the sarcomere length. The independence of the sarcomere length suggests that it cannot be any pH-induced lateral contraction. Since the drip loss is not related to the T_{23}-relaxation ($r=0.32^{n.s.}$) either, it is not likely that the difference in the water-holding capacity depends on the denaturation of myofibrillar proteins, giving rise to volume shrinkage of myofibrillar space and hence a lower T_{23}. We instead suggest that it is the aggregation of sarcoplasmic proteins that causes the loss in water-holding for normal meat. Von Seth *et al.* (1991) have shown that for normal meat (FOP-values <55) the solubility of sarcoplasmic proteins is reduced compared to that of DFD meat. Moreover, Lopez-Bote *et al.* (1989) found a continuous decrease in the solubility of sarcoplasmic proteins with higher light scattering for normal meat. The negative relationship (r=-0.71***) between the FOP-value and the pH for normal meat in Figure 6 further substantiates this observation. Von Seth *et al.* (1991) also observed that the loss of solubility of sarcoplasmic proteins explained most of the variation in light reflectance (FOP-values) for a studied material of normal and temperature-induced PSE pork muscle (LD).

Offer and Knight (1988) argue that they cannot see any explanation of how the denaturation of sarcoplasmic proteins can bring about a change in the water holding properties of meat. Our suggestion for the increase in drip loss due to the aggregation of the sarcoplasmic proteins in normal meat is the following. The sarcoplasmic proteins cannot pass the cell membrane as long as it is intact, which is probably the case most of the time during the rigor process. Von Seth and Tornberg (unpublished results) have found that the time-course of rigor (isometric tension) and the development of light scattering is parallel or quicker for the latter for normal and PSE meat. Being polyelectrolytes, proteins give rise to an osmotic effect, called the Donnan effect. If the sarcoplasmic proteins start to aggregate (observed as increased light scattering) the charges on the proteins become more shielded and the Donnan effect declines. This will give rise to a flow of water out of the cell before completion of rigor. When rigor is completed, however, the developed constrains, due to contraction, probably rupture the cell membranes partly and the sarcoplasmic proteins can then fill the extracellular volume created during rigor. The aggregated proteins most probably remain in the cell and the extra water outside the cell will not flow back, hence a higher drip.

CONCLUSIONS

Differences in the water holding of raw *longissimus dorsi* pork muscle of different meat quality can be detected using pulse-NMR measurements. The drip loss is governed by T_{22}-relaxation time, reflecting mainly the size of the channels outside the fibre bundles, from where the source of the drip emerges. The water-holding of the PSE meat was found to be governed by the aggregation of myofibrillar proteins, leading to contraction of the myofibrillar space and giving rise to a shorter T_{23}-relaxation time. For DFD meat it is the extent of low lateral shrinkage of the filament lattice induced by the high ultimate pH, that governs the properties of the meat. The difference in the water holding of normal meat seems to be governed by the aggregation of sarcoplasmic proteins.

REFERENCES

BENDALL, J.R., and WISMER-PEDERSEN, J. 1962. Some properties of the fibrillar proteins of normal and watery pork muscle. *J. Food Sci.* 27:144-159.

BORISOVA, M.A., and ORESHKIN, E.F. 1992. On the water condition in pork meat. *Meat Sci.* 31:257-265.

CLOKE, J.D., DAVIS, E.A., GORDON, J., HSIEH, S.Z., GRIDER, G., ADDIS, P.B., and McGRATH, C.J. 1981. Scanning and transmission electron microscopy of normal and PSE porcine muscle. *Scanning Electron Microscopy/III* SEM. Inc. AMF O'Hare, IL 60666, USA, 435-446.

EINSTEIN, A., 1956. *Investigations on the theory of Brownian Movement.* Dover.

FJELKNER-MODIG, S., and TORNBERG, E. 1986. Water distribution in porcine M. longissimus dorsi in relation to sensory properties. *Meat Sci.* 17:213-231.

HAMM, R. 1975. Water-holding capacity of meat. In: COLE, P.J.A., and LAWRIE, R.A. (eds). *Meat.* Butterworths, London. 321pp.

HONIKEL, K.O., and KIM, Ch.-J. 1986. Causes of the development of PSE pork. *Fleishwirtshaft.* 66:349-353.

KOTTER, L. 1955. *Neue wege bei der histologischen wurstuntersuchung.* Berl. Münch. Tierärtzl. Wschr. 68:360 pp.

LARSSON, G., and TORNBERG, E. 1988. An attempt to relate meat quality of pork (m.longissimus dorsi) to meat structure. *Proc. 34th ICMST.* Brisbane. 588pp.

LILLFORD, P.J., CLARK, A.H., and JONES, D.V. 1980. Distribution of Water in Heterogeneous Food and Model Systems. In: ROWLAND, S.P. (ed). *Water in polymers.* ACS Symposium series. 127:177pp.

LOPEZ-BOTE, C., WARRIS, P.D., and BROWN, S.N. 1989. The use of Muscle Protein solubility Measurements to assess pig lean meat quality. *Meat Sci.* 26:167-175.

NILSSON, R. 1969. Swedish Meat Research Institute, Anal. Instr. 6.

OFFER, G., and COUSINS, T. 1992. The mechanism of drip production: Formation of Two compartments of extracellular space in Muscle post-mortem. *J. Sci. Food Agric.* 58:107-116.

OFFER, G., and KNIGHT, P. 1988. The structural basis of water-holding in meat. In: (LAWRIE, E. (ed). *Developments in Meat Science.* 4:63-245.

OFFER, G., KNIGHT, P., JEACOCKE, R., ALMOND, R., COUSINS, T., ELSEY, J., PARSONS, N., SHARP, A., STARR, R., and PURSLOW, P. 1989. The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food Microstruc.* 8:151-170.

PENNY, I.F. 1977. The effect of temperature on the drip, denaturation and extracellular space of pork longissimus dorsi muscle. *J. Sci. Fd. Agric.*, 28:329-338.

RENOU, J.P., MONIN, G., and SELLIER, P. 1985. Nuclear magnetic Resonance Measurements on pork of various qualities. *Meat Sci.* 15:225-233.

RENOU, J.P., KOPP, J., GATELLIER, Ph., MONIN, G., and KOZAK-REISS, G. 1989. NMR-relaxation of water protons in normal and malignant Hypothermia-susceptible pig muscle. *Meat Sci.* 26:101-114.

von SETH, G., TORNBERG, E., and MÖLLER, B.-M. 1991). The influence of constant rigor temperature and storage time on the internal reflectance (FOP in pork muscle). Proc. 37th ICMST. Kulmbach, Germany. 3:40, 481-484.

SWATLAND, H.J. 1988. Effects of slaughter techniques on pig meat quality. Proc. from Pig carcass and meat quality. Reggio Emilia, Italy.

TORNBERG, E., and NERBRINK, O. 1984. Swelling of whole meat and myofibrils - as measured by pulse-NMR. *30th European Meeting of Meat Research Workers.* Bristol, UK. 3-10:112pp.

TORNBERG, E., and LARSSON, G. 1986. Changes in water distribution of beef muscle during cooking - as measured by pulse-NMR. *32nd European Meeting of Meat Research Workers.* Ghent, 9:4, 437-440.

TORNBERG, E., ANDERSSON, A. GÖRANSSON, Å., and von SETH, G. 1992. Water and fat distribution in pork in relation to sensory properties. OECD Seminar "Pork Quality, Genetics and Metabolic Factors", Helsinki.

Table 1. Raw meat quality and structural traits for the three meat quality groups normal, PSE and DFD. Mean values (x) and standard deviations (SD) are given.

	Normal X S.D.	PSE X S.D.	DFD X S.D.	Significance Level N-PSE	 N-DFD	 PSE-DFD
# samples	26	8	6			
Ultimate ph	5.45 0.15	5.41 0.12	6.18 0.18		***	***
FOP value	35.1 10.3	68.4 12.3	13.8 5.0	***	***	***
Drip loss (%)	2.7 1.0	4.4 1.4	1.0 0.3	***	***	***
Sarcomere length, µm	1.78 0.08	1.78 0.18	1.76 0.04			
Pulse-NMR T22, ms	131.9 17.9	145.0 18.0	98.4 14.5	p= 0.08	***	***
Pulse-NMR T23, ms	43.9 2.8	40.8 2.9	43.2 3.54	**		

1 Significant differences between meat quality groups:
**: $P \leq 0.01$; ***: $P \leq 0.001$.