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WATER DISTRIBUTION IN FRESH MEAT AS ASSESSED BY MRI-MEASUREMENTS AND ITS RELATION TO DRIP LOSS

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Introduction

The waterholding capacity of meat (drip loss) is an important quality attribute, since it affects consumer acceptance and the final weight of the product. Despite its economic importance, the mechanism of drip formation has not been completely revealed yet. A general hypothesis for the loss of drip of meat is that it is caused by shrinkage of the myofibrils, whereby fluid migrates to the extracellular spaces and gradually to the meat surface (Offer and Knight, 1988).

Various methods have been provided for assessing waterholding capacity, e.g. measurement of drip loss (Lundström and Malmfors, 1985) and fluid absorption by filter paper (Kauffman et al., 1986). However, these methods provide little or no information about the mechanism of fluid loss. With Magnetic Resonance Imaging (MRI) this problem may be overcome, because MRI provides a non-invasive and non-destructive determination of the quantity of water present and its relation to structure of biological materials. With this technique proton density and relaxation times (T1, T2) of different places in a sample can be mapped.

In general, in muscle more than 90% of the T2-relaxation decay can be described by 2 exponential rates: a short component (T2s) of 20-50 ms and a long component (T2l) of > 80 ms (Cole et al., 1993). However, it is still not clear where this bi-exponential decay originates from. Some authors suggested that different water compartments in muscle are characterised by their own T2, whereby it is often accepted that these water compartments are represented by the intra- and extracellular spaces of muscle. T2s is associated with intracellular and T2l with extracellular water (Cole et al., 1993; Hazlewood et al., 1974). There are strong indications for the validity of this theory. Addition of $MnCl_2$ to perfusion fluid of heart-muscle leads to differences in T2l, but not in T2s. As $MnCl_2$ migrates only very slowly into the fibres, this suggests that T2l reflects relaxation of extracellular water (Mauss et al., 1985). Another indication is that immediately after slaughter the % protons with T2l is hardly detectable, but develops after a certain time, probably after the development of rigor (Fung and Puon, 1981; Pearson et al., 1974). When muscle tissue is completely damaged only one T2 is found. However, after treatment of muscle with glycerine (damaging of the plasmalemma) a bi-exponential T2 was still observed (Cole et al., 1993). Lillford et al. (1986) showed that a physical barrier is not always necessary for a bi-exponential T2 to occur. They suggested that it originates from heterogeneity in the structure of a sample, such as is brought about by differences in size between extracellular and intracellular space.
In the present experiment MRI is used to study water distribution in fresh pork and beef in relation to its waterholding capacity (drip loss). Results are interpreted by the theory discussed above.

Material and methods

In the first experiment 4 pigs were selected on the basis of loin pH at 45 min. p.m. Two animals exhibited a fast pH-fall ($pH_{45} \leq 5.9$), whereas the others showed a normal pH-fall ($pH_{45} \geq 6.3$). pH was measured with a portable pH meter, equipped with a combined (glass/reference) electrode. The day after slaughter the *longissimus thoracis* was excised and divided in 4 parts of ± 300 g. After 7 days p.m. drip loss was measured according to Lundström and Malmfors (1985). At 2 days p.m. T2 of the meat was measured with a SISCO 200/400 *in vivo* NMR-spectrometer, equipped with active screened linear field gradients (max. 18 mT/m). T2-relaxation was observed using Carr-Purcell-Meiboom-Gill multi-echo sequence. Exication took place with a slice-selective 90°-pulse, while the spin-echo signal was refocussed repeatedly with non-selectieve 180°-pulses. Echotime was 2½ ms.

The second experiment involved 4 electrically stimulated (68 V, 14 Hz, 55 s) and 4 non-stimulated cow carcasses. The day after slaughter the same procedure as in the first experiment was used, however drip loss was measured at 14 days p.m.

Results and discussion

a. Pork. T2-relaxation in pork could be described by the following model: $y(\tau) = M_1 \exp(-\tau/M_2) + M_3 \exp(-\tau/M_4)$, where M_2 = T2s, M_4 = T2l, M_1 = relaxation-amplitude T2s, $_{M3}$ = relaxation-amplitude T2l. The percentage protons with T2s and T2l, P2s and P2l respectively were calculated by $P2s = 100*(M_1/M_1+M_3)$ and $P2l = 100*(M_3/M_1+M_3)$.

In Table 1 the results of the T2 measurements of pork are presented. About 80% of the muscle water was characterised by T2s, while the remaining part was described by T2l. This agrees with the percentage water in the myofibrils (80%) and the percentage free water in muscle (Offer and Knight, 1988). There was no relationship between drip loss and T2s. Differences in drip loss are coincided with differences in T2l and P2l. T2l increased with increasing drip loss (r=0.89). The relationship between T2l and drip loss can be explained in two ways. Drip loss is caused by shrinkage of the myofibrils during development of rigor, whereby the amount of extracellular water increases (Offer and Knight, 1988). T2l is believed to represent this fraction water in the muscle. The larger the amount of extracellular water, the larger T2l (Cole et al., 1993). Tornberg et al. (1993) suggested that T2 depends on the velocity of exchange between free and bound water molecules. This velocity depends on the diffusion distance and thus, like drip loss, on the size of the pores in the meat. The larger the pores the larger the amount of drip and the higher T2l.

P2l decreased with increasing drip loss (r=-0.75). It was expected that a high drip loss would result in a high P2l, because meat with a low water holding capacity is believed to have a large amount of extracellular water. The negative relationship in the present experiment can be caused by MRI-measurements having been conducted at 2 days p.m.. At that time meat with a low waterholding capacity, as used in this experiment can have already lost much (Honikel and Kim, 1985). Hence the amount of extracellular fluid is diminished and consequently P2l will be smaller than this is the case with a high waterholding capacity.

Table 1 pH at 45 min. p.m., Magnetic Resonance Imaging results p.m. of at 2 days p.m. and drip loss at 7 days p.m. of pork loins loss

no.	pH (-)	T2s (ms)	P2k (%)	T2l (ms)	P2l (%)	Drip (%)
1	6.27	26.6	61.3	56.6	38.7	5.6
2	6.31	31.2	81.1	70.1	18.9	7.7
3	5.78	31.5	82.3	71.5	17.4	8.6
4	5.80	31.8	88.0	80.6	12.0	16.5

Table 2 Magnetic Resonance Imaging results at 2 days different places in pork loin chops used for assessing drip according to Lundström and Malmfors (1985)

place	T2s (ms)	P2k (%)	T2l (ms)	P2l (%)
top	26.6	55.8	54.6[a]	44.2
middle	26.8	51.8	52.0[a]	48.2
bottom	24.3	45.3	42.0[c]	54.7

[abc]Values with different superscript differ significantly ($p<0.05$)

MRI provides a method for determining the macroscopical distribution of fluid within a sample. In Table 2 results of such a determination are presented. The place in the muscle influenced T2l. T2l of the bottom part of the muscle sample was shorter than T2l of the middle and top side. A shorter T2l indicates that the relative amount of water in the extracellular space is decreased. Therefore, it seems that more fluid was lost more rapidly from the bottem than from the other sides. This is likely caused by pressure differences within the muscle resulting from the sample's own weight.

b. Beef. In beef, T2-relaxation did not follow a bi-exponential pattern (Table 2). In most cases more than 97% of the relaxation of the muscle water could be described by T2s. Therefor it seemed more reliable to describe the relaxation proces by a mono-exponential model: $y(\tau) = M_1 + M_2 \exp(-\tau/M_3)$, whereby $M_3 = T2$. This suggests that in most samples hardly any extracellular water was present. As drip is formed mainly from fluid that is pressed to the extracellular space by shrinkage of the myofibrils (Offer en Knight, 1988), little extracellular water would indicate low drip loss. This is in agreement with the very low percentage drip as measured after 14 days p.m. (Table 3). Besides, the only muscle with a bi-exponential relaxation (no. 6) showed the highest drip loss.

In contrast with the experiment with pork no relationship between drip loss and T2l, P2l was found. This is probably caused by the extremely low drip losses (Table 3).

Table 3 Magnetic Resonance Imaging-results at 2 days p.m. and drip loss at 14 days p.m. of electrically stimulated (ES) and non-stimulated (NS) beef samples

ES sample	T2k[1] (ms)	P2k[1] (%)	T2l[1] (ms)	P2l[1] (ms)	T2[2] (ms)	Drip (%)	NS sample	T2k[1] (ms)	P2k[1] (%)	T2l[1] (ms)	P2l[1] (%)	T2[2] (ms)	Drip (%)
1	38.4	95.1	104.0	4.9	40.0	2.3	5	36.7	99.3	429.4	0.7	37.4	3.2
2	39.2	99.4	715.4	0.6	39.7	2.0	6	42.8	76.6	115.2	24.4	53.1	3.4
3	36.9	99.0	313.3	1.0	37.9	2.8	7	36.7	98.9	337.4	1.1	37.7	2.4
4	37.2	99.2	392.2	0.8	37.9	1.6	8	38.1	97.8	169.0	2.2	39.9	2.2

[1]Bi-exponential model, [2]Mono-exponential model

Conclusions

-MRI seems to be a valid method to detect differences in the distribution of water within a sample.
-The waterholding capacity of meat seems to be characterised by the long component of the T2-relaxation (T2l) as measured by MRI.
-Meat with high drip losses has a longer T2l than meat with low drip losses. The percentage of protons with a long relaxation time is hardly detectable in meat with an extremely high waterholding capacity. Therefore drip loss seems to be strongly related to the amount of extracellular fluid present in the sample. To assure of correct interpretation of the results more information about the relationship between MRI-parameters and the structure of muscle is necessary.

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