13

The Identification of Normal and Watery Pork by Pulsed Nuclear Magnetic Resonance Measurements

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INTRODUCTION

The occurrence of PSE meat /pale, soft, exudative/ causes considerrable problems in meat technology. This meat has an atypical course of ripening and has unsuitable technological properties leading to economic losses. The incidence of PSE meat is governed by genetic factors and intensive fattening of animals. The disadvantage in question concerns mainly the most important carcass element, longissimus dorsi, and some of ham muscles.
The PSE meat differs from normal by its lighter, pale colour, very small water holding capacity after the slaughter, and nonadherent, soft structure. All these features considerably limit the technological usefulness of PSE meat.
Fast identification processed meat would allow to avoid many losses caused by decreased efficiency of products. The PSE meat may be identified by various methods. They are based on meat colour /Janicki et al. 1967/, amount of bound water /Grau and Hamm, 1952/, Hart's test /Hart, 1962/, hardness and consistence /Briskey et al., 1962/, solubilization of muscle proteins /Bendal and Wismer - Pedersen, 1962/, ATP to IMP ratio /Fischer and Augustini, 1977/ etc. However, as these methods are time - and - labour - consuming and sometimes insufficiently accurate, they are not applicable when fast and simple assays are required.
The most widely used method of detecting the watery structure of muscle seems to be pH measurement about 45 minutes after slaughter /Briskey and Wismer - Pedersen, 1961/.
However, in recent times many authors have held the opinion that the measurement of that value does not always suffice to classify watery and normal pork /Blendl and Puff, 1978; Barton, 1980; Lengerken and Hannerback, 1979; Pfutzner and Fialik, 1982/.
As far as the authors know, there has been little research done on muscles using pulsed nuclear magnetic resonance /Renou at al. 1984; Tornberg E, Nerbrink ". 1985/.
This technique, however, has been used to fat /Madison and Hill, 1978/, protein /Tipping, 1982; Wright, 1980/ and H_2O content determination /Weisser, 1980; Brosio, 1982/. Pulsed nuclear magnetic resonance studies have been done as well to differentiate normal and pathological tissues /Adamski at al. 1983; Koutscher at al. 1978/ and to follow the post mortem changes /Chang at al., 1976/.
In this work the authors would like to find out wheter pulsed NMR could be used to identify normal, intermediate and watery pork of longissimus dorsi.

MATERIAL AND METHODS

Test were made after 2, 24, 48 hours after slaughter on longissimus dorsi originating from pigs of 90 - 110 kg. About 45 minutes later, pH_1 of the carcass examined was measured using pH - meter of N 5111 type /MERA - ELWRO/. Muscle classification was performed on the basis of pH measurements and R values /ATP/IMP/. Using the following criteria: $pH > 6.3$ - normal muscles $6.3 > pH > 6.0$ - intermediate watery muscles /IPSE/, and $pH < 6.0$ - watery muscles /PSE/. The muscle samples were extracted from between the 9 th at 11 th rib. From each muscle samples weighing 500 mg were collected. The samples were transfered in closed test - tubes at 277 K to NMR laboratory. The measurements of relaxation times were performed on a pulsed NMR spectrometer operating at 27 MHz. In our research we utilized the absorption of radiofrequency waves by the proton magnetic moments in the static magnetic field. The relaxation time T_1 /spin-lattice/ was determined by $180^\circ - \tau - 90^\circ$ method, and the relaxation time T_2 /spin-spin/ by Carr - Purcell - Meiboom - Gill method. The measurements were carried out at 298 - 1 K and stabilized by continuous nitrogen flow. Because of small /less then 5 %/ differences between relaxation times for samples of the same muscle, results were averaged.
In this work the fallowing simple arithmetic formula which allows to compare, in the same scale, all deviations from mean normal values for examined samples was used:

$$Q = \frac{/T_{1i} - \bar{T}_{1N}/}{\bar{T}_{1N}} + \frac{/T_{2i} - \bar{T}_{2N}/}{\bar{T}_{2N}} \qquad /1/$$

where; $T_{1,2i}$ are relaxation times T_1 and T_2 of particular samples, respectively, and $\bar{T}_{1,2N}$ are mean relaxation times T_1 and T_2 of normal muscle, respectively.
If T_1 and T_2 of studied sample are the equal of mean relaxation times of normal muscle then Q - value will be zero. Every Q greater than 0 states a deviation from normal values of T_1 and T_2.
Formula /1/ allows for simultaneous comparison of relaxation time differences between sample and mean values in the same scale. It is a useful approach if we recall that T_1 is of order of 600 ms and T_2 - of 40 ms in studied muscle.

RESULTS

The results of measurements of relaxation time T_1 and T_2 in longissimus dorsi samples, done 2 hours after slaughter are shown in Fig 3 and 4, respectively. The mean values of T_1 and T_2 for studied muscles at various time intervals after slaughter are presented in Figs 1 and 2.
Fig. 5 shows distribution of Q values calculated for relaxation times measured 2 hours after slaughter. Table 1 presents the mean values of T_1, T_2 and Q, whereas table 2 the differences between T_1 and T_2 of the examined muscles.

TABLE 1
Mean values of T_1, T_2 and Q for the samples 2 hours after slaughter

Type of muscle	pH_1	$\bar{T}_1$ /ms/	N	S-D	t-s	$\bar{T}_2$ /ms/	S-D	t-s	Q	S-D	t-s
NORMAL	pH > 6.3	633	21	0.02	-	29.0	0.004	-	0,146	0.07	-
IPSE	6.0-6.3	603	17	0.02	x x	25.7	0.003	x	0.157	0.09	NS
PSE	pH < 6.0	569	25	0.03	x x	25.2	0.003	x x	0.270	0.06	x x

N - number of cases; SD - standard deviation; Q - value was calculated according to formula 1, t-s - results of t-Student test; x, x x - statistically significant differences at 0.05 and 0.01 level; NS - differences statistically non - significant.

TABLE 2
Differences in relaxation times of examined muscles. $T_{1,2i}$ - relaxation times T_1 and T_2 of studied samples; $T_{1,2N}$ - mean relaxation times T_1 and T_2 of normal muscles; Q - value was calculated according to formula /1/

Type of muscle	time after slaughter	$T_{1i} - \bar{T}_{1N}/\bar{T}_{1N}$	$T_{2i} - \bar{T}_{2N}/\bar{T}_{2N}$	Q
Normal	2	0.020	0.126	0.146
	24	0.033	0.075	0.105
	48	0.045	0.131	0.177
IPSE	2	0.047	0.114	0.157
	24	0.049	0.021	0.110
	48	0.054	0.107	0.155
PSE	2	0.098	0.121	0.270
	24	0.126	0.083	0.210
	48	0.116	0.062	0.241

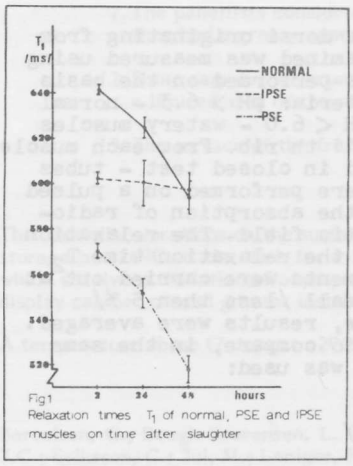


Fig1
Relaxation times T_1 of normal, PSE and IPSE muscles on time after slaughter

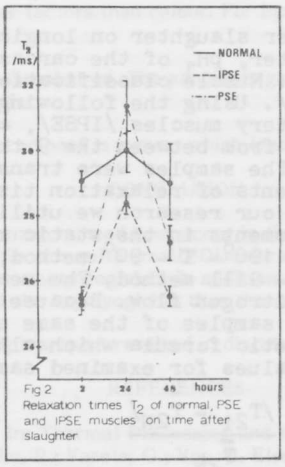


Fig 2
Relaxation times T_2 of normal, PSE and IPSE muscles on time after slaughter

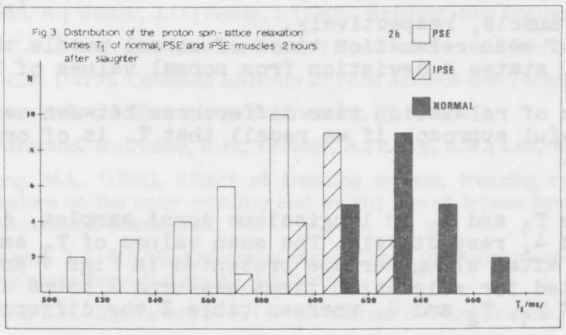


Fig 3 Distribution of the proton spin - lattice relaxation times T_1 of normal, PSE and IPSE muscles 2 hours after slaughter

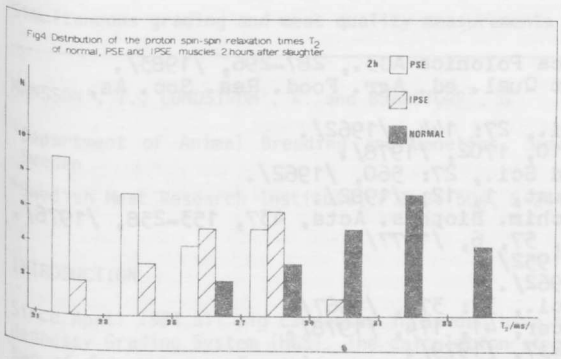
DISCUSSION

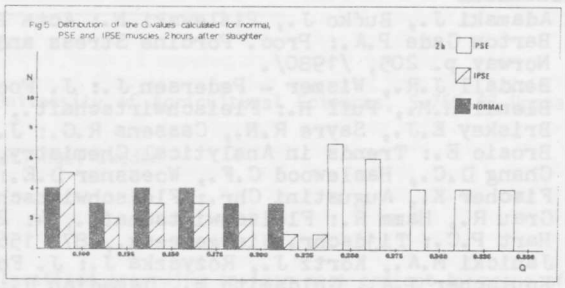
T_1 values

The longest values of spin-lattice relaxation time T_1 were observed in normal muscles with pH_1 greater than 6.3. The relaxation times of normal samples are always longer than 640 ms /fig. 1/. The shortest T_1 were found in watery muscles, in all cases below 570 ms. Intermediate muscles, with pH within 6.0 - 6.3, possess T_1 around 600 ms. The differences between normal and watery muscles in relaxation times T_1 are statistically valid /$p < 0.01$/ /Tab. 1/.
24 hours after slaughter, a decrease of T_1 in all types of muscles /Fig. 1, tab. 1/ was observed, the decrease for PSE being the biggest.
48 hours after slaughter further decrease was seen. The most important fact, however, is that the T_1 differences between normal and watery muscles increased by about 80 ms in this time.

T_2 values

Similarly as for T_1, the spin-spin relaxation times T_2 are statistically different /$p < 0.01$/ between normal and PSE muscles /fig. 2, tab. 1/. The T_2 differences between normal and intermediate muscles /IPSE/ are statistically different to, at 0.05 level. 24 hours after slaughter an increase of T_2 /fig. 2/ was observed, as opposed to T_1 changes. 48 hours after





slaughter a decrease of T_2 was visible, to the values close to those measured at 2 hours.

Q values

Calculated Q values are the biggest for PSE muscles /tab. 1/ 2 hours after slaughter. At this time Q values for IPSE are close to those of normal muscles. 24 hours after slaughter Q for IPSE is somewhat longer than Q for normal muscles but, again, the highest Q values are calculated for PSE.
Similar effect is observed 48 hours after slaughter: Q for PSE has the longest values, although smaller than at 2 hours. Table 2 enables one to put the differences between relaxation times of studied muscles into one scale. Otherwise it would be impossible to compare directly the T_1 differences /e.g. $633_N - 569_{PSE} = 64$ ms/ and the T_2 differences /e.g. $29_N - 25_{PSE} = 4$ ms/, 64 ms versus 4 ms.
Thus, for example, as it is seen in table 2, the T_1 differences between normal and IPSE meat are actually stable in time, whereas the T_2 differences are the largest 2 hours after slaughter the Q results presented in the same table reveal for bigger values, because Q cimprises the differences in T_1 and in T_2.
In our view, the formula /1/ used in this work is better than another, proposed by Koutcher /1978/ and used originally to study normal and pathological tissues.
Formula /1/ first counts the differences between the sample and the mean, in plus or in minus, then normalizes the T_1 differences and the T_2 differences into one scale.

Normal versus PSE muscles

The biggest differences between normal and PSE muscles are seen 2 hours after slaughter when the Q - value is calculated /fig. 5, tab. 1/. It has been noticed, as well, that T_1 alone is quite a good indicator of muscle type /fig. 1/.
In the case of T_1, however, there is an overlapping between these types of muscles /fig. 3/. 24 hours after slaughter the Q and T_1 values are also different for normal and watery muscles.
The T_2 relaxation time could be used as a muscle indicator 2 or 48 hours after slaughter for at 24 hours it has similar values for all types of muscles /fig. 2/.
The best means to differentiate normal and watery muscles at 48 hours after slaughter seems to be the measurement of T_1 and, consequently, the calculation of Q /fig. 1, tab. 2/.

Post mortem changes

Several authors have used NMR to study post mortem changes in muscles. Chang et al /1976/ observed elongation of T_1 in rat skeletal muscle. The longest values were measured about 3 hours after sample removal. After that about a 30 % drop to a stable level was observed.
Adamski et al. /1983/ reports that the T_2 changes in human uterus muscle follow the same pattern.
At the beginning, within 2 hours, the T_2 increase is very fast, then reaches a stable level.
We understand that this work is the first report of simultaneous measurements of T_1 and T_2 time dependence in technological meat /figs 1, 2/. We suggest that the T_1 and T_2 changes reflect distinct processes. As it is seen in tab. 1, muscles with high pH have longer relaxation times. Only T_1, however, exhibits such changes /fig. 1/ T_2 values seem to indicate processes reaching their maximum about 24 hours after slaughter.
There is good consent between the T_2 changes and course of rigor mortis. When T_2 increases and then drops, rigor mortis reaches its maximum and vanishes. It should be, as well, stressed that 24 hours after slaughter the biggest autflow of water from muscles is observed.
The increase in free water fraction should result in elongation of relaxation times.
PSE muscles have the biggest water outflow and the longest T_2 values 24 hours after slaughter. It is hat, however, clear why this effect is not observed in T_1 as well.

CONCLUSIONS

1. The measurement of T_1 2 hours after slaughter is enough to perform fast identification of longissimus dorsi by means of pulsed NMR. This is the time of the largest differences between relaxation times of normal and PSE muscles.
2. The best differentiation of normal and PSE muscles could be achieved by the calculation of Q - value 2 or 24 hours after slaughter.
3. To study post mortem changes, measurements of T_1 and T_2 are necessary for they seem to reflect different effects concerned with this process.

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