WATER MOBILITY DURING RIGOR MORTIS OF PORCINE MUSCLE WITH DIFFERENT PH FALLS

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Abstract –The water mobility in porcine muscle was investigated during rigor mortis in response to fast and normal pH decline. Low-field NMR T2 relaxometry parameters were measured accompanying with meat quality attributes measurement. The pH fall showed a significant effect (P<0.0001) on T2 components, in which relax time of bound water (T2b) was higher for the fast group than that of the normal group. Populations of bound water and immobilized water (P2b and P21) of the fast group were kept lower than that of the normal group, but it was reverse for the population of free water(P22), indicating that a greater water mobility occurred in the fast pH fall muscles. L* and b* values were significantly different between the two groups after 5h postmortem (P<0.05), but a* value did not change significantly (P>0.05). It is notable that the difference in P21 and P22 between the two groups appeared as early as 3h postmortem, which could be a good indicator for fast detecting PSE/RSE meat.

Key Words – PSE meat, Low-field NMR

I. INTRODUCTION

The water holding capacity (WHC) is a major quality attribute of fresh meat as it determines potential storage loss, technological quality and appearance of fresh meat [1]. Meat with poor WHC is regarded as soft and exudative meat, which may become pale or keep reddish. Moreover, WHC may affect the textural properties of the cooked meat [2]. The WHC of meat was dependent on the mobility of water during the conversion of muscle to meat.

The muscle pH in a rigor state has been shown an indicator for meat quality [3]. Generally, meat could have the characteristics of pale or reddish, soft and exudative (PSE or RSE) meat if muscle pH value is lower than 6.0 at 45min postmortem, whilst it could the characteristics of dark, firm and dry (DFD) meat if muscle pH is still higher than 6.0 at 24h postmortem. To a certain extent, the incidence of PSE meat is associated with environmental temperature and its fluctuation, in particular for an extremely high incidence of PSE meat in summer.

NMR relaxation measurements have been shown to correlate with muscle pH decline at early postmortem time. Previous studies indicated that T_2 components of pork at 24 h postmortem were associated with pH decline and ultimate pH, in which the ultimate pH of pork had a negative or positive relationship with loosely bound myowater [4]. However, there are still some discrepancy in the relationship between ultimate pH and water distribution. Moreover, relaxation T_2 components of different pH falls pork has not been paid much attention during rigor mortis before 24 h postmortem.

Therefore, the present study was designed to investigate the changes of relaxation measurements in porcine muscle during rigor mortis and subsequent aging and their associations with pH decline and other pork quality.

II. MATERIALS AND METHODS

2.1. Sampling

A total of 30 Chinese native breed pigs were randomly selected from a local slaughterhouse 30 km far from the laboratory. At approximately 45 min after bleeding, *longissimus dorsi* muscle between the last three lumbar vertebrae was removed and center temperature and pH of meat and the color of its cutting surface were measured as the initial measurements. After that, the samples were individually packed, placed on ice and transported to the laboratory.

After transported to the laboratory, the samples were chilled and stored at 0-4℃ till 48h postmortem. The temperature, pH, meat color and LF-NMR of muscle samples were measured at 3, 5, 7, 9, 12, 24 and 48 h postmortem. The samples were cooked and sheared at 48h postmortem.

2.2 pH/temperature measurements and grouping

The pH of pork samples were measured in triplicate using a combination puncture electrode (Mettler Toledo) and the center temperature of meat samples were recorded in triplicate using a Testo temperature probe (Testo108,Testo AG, Germany). The 30 samples were divided into two groups by the initial pH (pH45min), i.e., Group A in which pH45min was greater than 6.0) and Group B in which pH45min was smaller than 6.0.

2.3 NMR transverse relaxation (T2) measurement

NMR relaxation measurement was performed as previously described [5] with minor modification. Briefly, three 1.5×1×1 cm strips were removed along the fiber direction from each *longissimus dorsi* sample. The strips were individually placed in a cylindrical glass tube (18 mm in diameter). Put a plastic film on each tube's mouth to avoid moisture volatile. The tubes were inserted into the probe of a Niumag Pulsed NMR analyzer (PQ001, Niumag Corporation, Shanghai, China) one by one. The analyzer was operated at a resonance frequency of 22.4 MHz at 32 °C. Transverse relaxation (T_2) was measured using the CPMG sequence with a τ-value of 200 μs. Samples were repeatedly scanned for 20 times at a 1 s interval. A total of 3000 echoes were acquired and fitted with a multi-exponential model under the program MultiExp Inv Analysis (Niumag Corporation, Shanghai, China). Three relaxation times (T_{2b}, T_{21}, T_{22}) and their corresponding population (P_{2b}, P_{21}, P_{22}) were recorded.

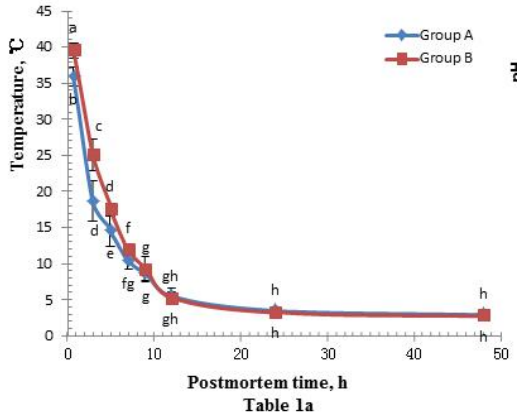
2.7 Statistical analysis

The effects of initial pH and storage time on NMR measurements were evaluated using a mixed-model analysis of variance, where the measured variables were set as dependent variables, time, group and time × group as fixed effects and sample number as random effect. The pairwise differences between least-square means were evaluated by the Bonferroni's method using the SAS program9.1.2 (SAS Institute Inc., Cary, NC, USA, 2003).

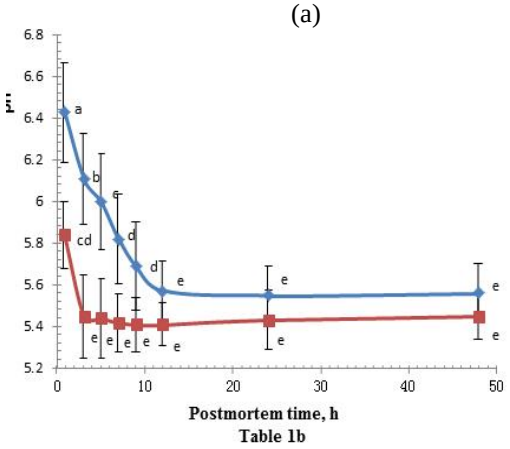
III. RESULTS AND DISCUSSION

3.1 pH and temperature fall

Based on initial pH (pH_{45min}), 22 samples were categorized into Group A (pH_{45min} greater than 6.0) and 8 samples were categorized into Group B (pH_{45min} smaller than <6.0). There was no sample with the pH value greater than 6.0 at 24h, indicating no incidence of DFD meat. The temperature of Group B was significantly greater ($P<0.001$) than that of Group A at 45min, 3h, and 5h postmortem, but there was no difference afterwards ($P>0.05$, Fig.1a). The pH value of Group B was significantly smaller than that of Group A within the first 12h but the difference was not significant afterwards ($P>0.05$, Fig.1b).



(a)



(b)

Figure 1 Temperature and pH decline
A for samples that had a normal pH decline, B for samples that had a fast pH decline

3.2 LF-NMR relaxation characteristics

Independently of the two groups, three components were detected, including a minor component between 1 and 5ms (T_{2b}), a major component between 10 and 100 ms (T_{21}), and a third component between 100 and 300 ms (T_{22}). The relaxation time and populations of T_{2b}, T_{21} and T_{22} were highly dependent on pH decline (Table 1). T_{2b} had an increase ($P<0.05$) with storage time, and T_{2b} of Group B was significantly

greater than that of Group A at each time point (3h to 48h, $P<0.05$). T_{21} of Group A increased within the first 9h postmortem but declined slightly afterwards. However, T_{21} continued to decrease with time in Group B ($P<0.05$). For T_{22}, Group B was smaller than that of Group A at 3h ($P<0.05$) and both groups had a trend to increase at early time that is followed by a decline.

Bound water (represented by P_{2b}) had a trend to decline in both groups, Group B was significantly smaller than group A till 48h postmortem. P_{21}, the population of immobilised water, remained constant till 9h postmortem and then declined in Group A, whilst it started to decline at 3h postmortem in Group B ($P<0.05$). The P_{21} value of Group B was smaller ($P<0.05$) than that of Group A. When it came to P_{22}, the population of free water, both groups had a trend to increase from 3h to 24h postmortem ($P<0.05$), but P_{22} of Group B kept significantly greater ($P<0.05$) than that of Group A across the storage time.

Principle component analysis of 3h and 24h samples indicated that the first two principle components (PCs) explained 60.93% and 73.35% of variation in all T_2 variables (T_{2b}, T_{21}, T_{22}, P_{2b}, P_{21}, P_{22}) (Figs 2 & 3). At 3h postmortem, samples in Groups A and B could not be well separated. However, they were separated at 24h, in which samples in Group A were collected at the lower left side and samples in Group B were collected at the upper right side. This indicated that the significant difference in water distribution in pork was highly associated with pH decline.

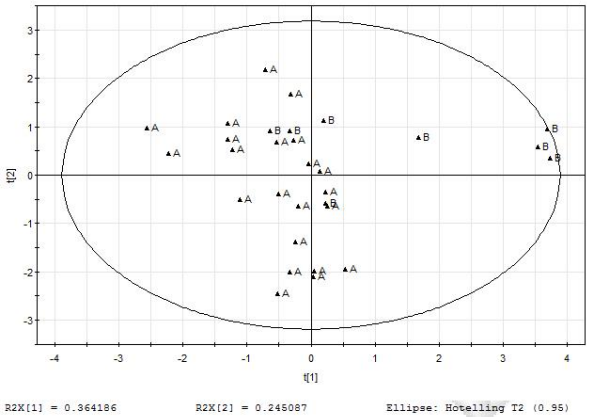


Figure 2 PCA analysis of the T2 relaxation attributes for the two groups at 3h postmortem
A for samples that had a normal pH decline, B for samples that had a fast pH decline

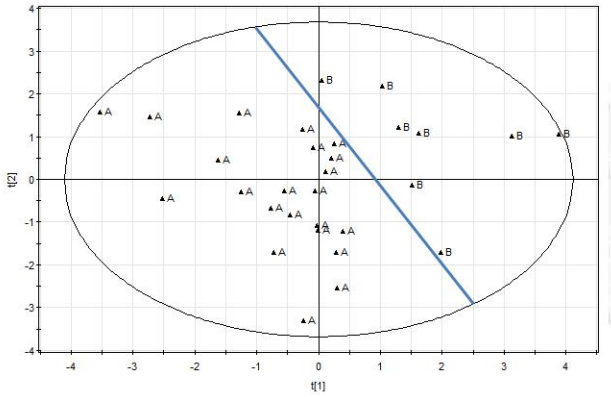


Figure 3 PCA analysis of the T2 relaxation attributes for the two groups at 24h postmortem
A for samples that had a normal pH decline, B for samples that had a fast pH decline

LF-NMR T_2 relaxation has been shown as a good approach to track the water distribution and mobility of fresh pork [6]. In the present study, we for the first time observed that the water mobility of pork, based on LF-NMR transverse relaxation measurement, was dependent on pH decline. T_{2b} of muscle samples with fast pH decline (Group B) was greater than that of normal samples (Group A), which could be in that the dramatic pH fall at early postmortem time could cause the serious denaturation of muscle proteins (especially of myofibrillar proteins) and the decreased capacity of binding water. Accompanying with pH decline, the capacity of binding water decreased with storage time for both groups. Accordingly, the T_{2b} population was greater for the samples with normal pH decline and it decreased with storage time.

T_{21} and T_{22} were not significantly different between the two groups. Unexpectedly, standard deviations were much great for each subgroup, indicating that the variations between individual samples overwhelmed the main effect of pH on the capacity of holding immobilized water and free water. However, for both groups, T_{21} had a positive response to pH decline with increased storage time, but it was negative for T_{22}. In contrast, P_{21} decreased but P_{22} increased with pH decline, indicating the movement of water from the intra/inter-myofibrils to extra-myofibrils. The P_{21} value of Group A at 12 h equals that of Group B at 3 h. This could be associated with the contraction of muscle sarcomeres [4]. In the subsequent storage, P_{21} continued to decrease,

which could be due to protein degradation, i.e., postmortem aging [3]. In addition, low pH might destroy the structure and steady state of the myofibrillar protein matrix [7].

IV. CONCLUSION

In the present study, porcine muscles were categorized into normal pH decline group (A) and abnormally fast decline group (B). The samples with a fast pH decline (Group B) had a poor WHC. T_{2b} of Group B was greater than Group A at 3h postmortem, showed a weak capacity of water holding around myofibrillar protein. A part of T_{2b} population became T_{22} population directly during rigor mortis. T_{21} and T_{22} were not significantly different between the two groups. It is notable that the difference in P_{21} and P_{22} between the two groups appeared as early as 3h postmortem, which could be a good indicator for fast detecting PSE/RSE meat.

ACKNOWLEDGEMENTS

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Table 1 Changes of T_{2s} relaxation attributes during the rigor and aging period[1]

		Postmortem time, h						
	Group	3	5	7	9	12	24	48
T_{2b}	A	1.22 ± 0.13^{d}	1.24 ± 0.17^{d}	1.25 ± 0.15^{d}	1.27 ± 0.17^{cd}	1.37 ± 0.19^{c}	1.44 ± 0.17^{bc}	1.51 ± 0.13^{b}
	B	1.46 ± 0.19^{bc}	1.48 ± 0.15^{bc}	1.51 ± 0.18^{b}	1.54 ± 0.12^{b}	1.59 ± 0.17^{ab}	1.72 ± 0.21^{a}	1.74 ± 0.22^{a}
T_{21}	A	43.58 ± 5.27^{b}	43.8 ± 5.13^{b}	44.82 ± 2.77^{ab}	45.5 ± 3.10^{a}	44.91 ± 2.83^{ab}	42.41 ± 2.30^{bc}	43.24 ± 1.55^{b}
	B	45.65 ± 4.03^{a}	44.51 ± 3.58^{ab}	44.34 ± 3.91^{ab}	44.19 ± 3.68^{ab}	43.58 ± 3.18^{b}	42.34 ± 3.05^{bc}	41.63 ± 2.61^{c}
T_{22}	A	192.5 ± 92.88^{ab}	187.85 ± 83.03^{ab}	208.98 ± 75.43^{a}	206.32 ± 87.48^{ab}	186.9 ± 86.51^{ab}	152.44 ± 30.95^{b}	154.36 ± 24.56^{b}
	B	156.51 ± 33.58^{b}	172.73 ± 44.77^{b}	168.73 ± 69.68^{b}	219.72 ± 62.28^{a}	170.42 ± 44.76^{b}	153.81 ± 19.7^{b}	158.29 ± 20.82^{b}
P_{2b}	A	3.59 ± 0.53^{a}	3.51 ± 0.57^{a}	3.40 ± 0.54^{a}	3.32 ± 0.42^{b}	3.24 ± 0.36^{b}	3.23 ± 0.41^{b}	3.13 ± 0.41^{bc}
	B	3.33 ± 0.33^{bc}	3.15 ± 0.29^{bc}	3.04 ± 0.38^{bc}	3.01 ± 0.30^{c}	3.00 ± 0.39^{c}	2.99 ± 0.33^{c}	2.96 ± 0.37^{c}
P_{21}	A	94.22 ± 0.67^{a}	94.20 ± 0.56^{a}	94.00 ± 0.62^{a}	93.90 ± 0.67^{ab}	93.58 ± 0.90^{c}	91.64 ± 0.94^{f}	91.45 ± 0.72^{f}
	B	93.28 ± 0.98^{c}	93.13 ± 1.08^{cd}	92.72 ± 1.06^{d}	92.30 ± 1.04^{d}	92.06 ± 1.16^{e}	90.25 ± 1.29^{g}	89.96 ± 1.01^{g}
P_{22}	A	2.19 ± 0.43^{f}	2.32 ± 0.30^{f}	2.50 ± 0.52^{ef}	2.78 ± 0.59^{e}	3.20 ± 0.89^{de}	5.16 ± 0.87^{b}	5.46 ± 0.69^{b}
	B	3.40 ± 1.12^{d}	3.60 ± 1.10^{d}	4.01 ± 1.12^{d}	4.18 ± 1.01^{d}	4.90 ± 1.01^{c}	6.80 ± 1.11^{a}	7.10 ± 0.96^{a}

[1] T_{2b}, T_{21}, T_{22}, relaxation times for water closely associated with macromolecules, water located within the myofibrillar protein matrix, and extramyofibrillar water, respectively;

P_{2b}, P_{21} and P_{22}, populations for water closely associated with macromolecules, water located within the myofibrillar protein matrix, and extramyofibrillar water, respectively;

^{a,b,c} Means on the same attribute (T_{2s} or P_{2s}) with different letters are significantly different ($P<0.05$).