The effect of brine concentration on diffusion and water distribution in pork muscles: a low-field nuclear magnetic resonance study

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***Abstract*— The water-holding capacity (WHC) of meat has a direct impact on its economic value. This study aims to assess the impact on WHC of different brine concentrations and-fibre directions and to assess the water mobility and distribution using novel and conventional methods. The novel method employed was low-field nuclear magnetic resonance (LF-NMR) which is a rapid and non-invasive technique that can be used for the analysis of water-hydration properties. Pork *M. longissimus thoracis et lumborum* samples were cured with varying brine concentrations (6, 18 or 36% NaCl) and fibre directions (parallel or perpendicular). Brine concentration had an effect ($p<0.05$) on all parameters examined, while fibre direction only had an effect on samples cured with 36% NaCl. LF-NMR results indicated that 6% NaCl caused increased intra-cellular water, 18% NaCl caused increased extra-cellular water and 36% NaCl dehydrated the sample of both intra- and extra-cellular water. WHC increased with 6% NaCl, but decreased with 18% and 36% NaCl. Moisture diffusion in the sample cured with 6% NaCl proceeded at a rate of 2.74×10^{-9} m^2/s, while higher NaCl concentrations resulted in moisture loss. Histological analysis supported the trend showing visible fibre swelling in samples cured with 6% NaCl, large inter-fibrillar spacing in samples cured with 18% NaCl and dehydrated structures in samples cured with 36% NaCl. These findings suggest that LF-NMR is a novel technique which can provide key information about water compartmentalisation in meat and that 6% NaCl is optimum for increased intra-cellular water entrapment and increased WHC.**

Keywords— **Water holding capacity, nuclear magnetic resonance, curing.**

I. INTRODUCTION

The interactions of meat, NaCl and water have been studied for many decades [1,2] due to their effects on textural, water holding properties and economic value of meat [3]. Low-field NMR is a novel technique which can provide further information on intra-/extra-cellular water in meat. Although many studies have been conducted using LF-NMR on cured meat [4,5], the effect of sodium chloride concentration and fibre direction on water holding capacity has not been studied. Also, the theory that relaxation behaviour of meat is related to water compartmentalisation is challenged by two other theories. The first suggests that the behaviour is related to the degree of fibre contraction [6] while the second suggests that it is related to fast exchange of water through membrane pores [7]. This study aims to give further insight into these theories and to assess if LF-NMR can provide further information on the optimisation of meat WHC through brine concentration and fibre direction.

II. MATERIALS AND METHODS

A. Meat sampling and treatment

Pork *M. longissimus thoracis et lumborum* muscles were obtained at 72 h post-mortem. Cylindrical samples (35ϕ × 25 mm) were cut parallel or perpendicular to the fibre direction and inserted into one end of polyethylene tubes (34ϕ × 130 mm) followed by a rubber stopper. The tubes were then inverted so that the meat and stopper were on the bottom and a brine solution (65 ml) containing 6, 18 or 36% NaCl was poured into the open end of the tube such that it came in contact with the exposed meat surface and the tube was covered. The samples were cured at 4°C for 22 h for LF-NMR, WHC or histological analysis. Samples for calculation of diffusion coefficients were cured and collected at intervals of 0, 1, 3, 5, 7 and 9 h. A non-treated pork sample acted as a control.

B. NMR measurement

The top 10 g of the cured sample was analysed using a Maran Ultra (Oxford Instruments, Abingdon, UK) low resolution NMR spectrometer with a

magnetic field strength of 0.5 Tesla and a resonance frequency for protons of 23.4 MHz. Transverse measurements (T_2) were conducted using the Carr-Purcell-Meiboom-Gill method [8,9]. The parameters for measurement were a τ value of 150 µs. Each measurement was from 16 scan repetitions.

The data were analysed by applying multi-exponential fitting of the T_2 relaxation data using RI Win-DXP programme (Oxford Instruments Molecular Biotools Ltd., Abingdon, UK). Three measurements were carried out on each sample. Further data analysis was carried out using Sigmaplot software (Version 11, Systat Inc. USA) which allows calculations of relaxation times, curve widths (CW) and area which acted as indications of water-binding, water homogeneity and component size, respectively.

C. Moisture analysis

Moisture analysis was performed by a Smart Trac 5 (Model 907875, CEM Cooperation, NC, USA).

D. Diffusion coefficients

Diffusion coefficients were carried out for moisture diffusivity according to a modification of Fick's second law [10].

E. Water holding capacity

Pork samples were cooked in a water bath (90°C, 10 min) and centrifuged (1000 rpm, 4°C, 10 min) in bespoke centrifuge tubes [11]. The WHC was calculated as percentage weight change before cooking and after centrifugation.

F. Histology

Pork samples (3 × 3 × 3 cm) were cured for 22 h by immersion in the assigned brine. The centre 1 × 1 × 1 cm was frozen in isopentane cooled in liquid nitrogen. Samples were held at -25°C and sliced to 20 µm thickness in a cryostat (Leica 1950 Cryostat, Leica Microsystems AG, Wetzlar, Germany). Samples were stained with toluidine blue, air dried and examined with a Leica DMLB light microscope (Leica Microsystems AG, Wetzlar, Germany).

G. Statistical analysis

A two-way analysis of variance (ANOVA) was carried out with factors of NaCl concentration and fibre direction. Muscle was a random effect. Principle component analysis (PCA) was performed for all NMR and WHC variables. All data were analysed using XLStat (XLSTAT version 2011.2 Addinsoft).

III. RESULTS AND DISCUSSION

A. NMR

Table 1. Correlations of LF-NMR variables with WHC

Variable	Significance	Correlation
T_{2b} ms	$P \leq 0.001$	-0.862
T_{21} ms	$P \leq 0.001$	0.787
T_{22} ms	$P \leq 0.05$	-0.668
T_{2b} area		-0.341
T_{21} area	$P \leq 0.001$	0.865
T_{22} area	$P \leq 0.05$	-0.785
T_{2b} CW		-0.364
T_{21} CW		0.542
T_{22} CW	$P \leq 0.05$	0.64

Three peaks were identified in the NMR results. A theory of water compartmentalisation has been hypothesised which suggests that the minor component (T_{2b}: 2-5% at 6-13 ms) is hydrogen tightly bound to macromolecules, the major and intermediate component (T_{21}: 86-92% at 39-54 ms) is intracellular hydrogen and the most loosely bound component (T_{22}: 5-9% at 133-177 ms) is extra-cellular hydrogen [12].

T_{2b} ms increased with increased NaCl concentration ($p<0.001$). This may be attributed to the increased osmotic stress. The opposite was observed for the T_{21} relaxation times, which decreased with increasing NaCl concentration. Similar findings were noted by authors who suggested that the increase in T_{21} relaxation times is due to the increase in water entrapment [12] and increased swelling of fibres, therefore a greater distance between myofibrils [4]. 5.8% NaCl has been reported to cause optimum fibre swelling [8, 13] and the increase in T_{21} ms and T_{21} curve area are indicative of this.

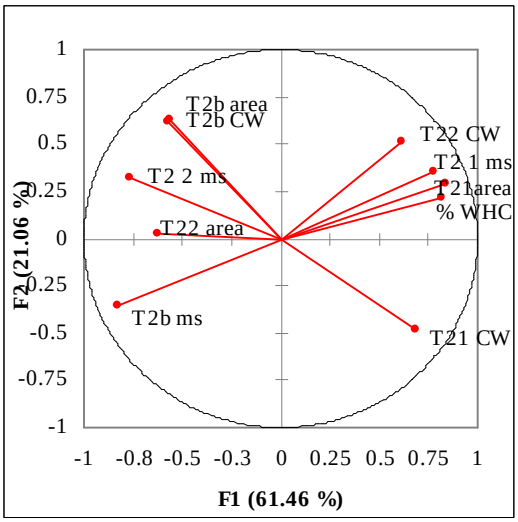

Fig. 1. PCA vector plot of WHC and LF-NMR variables

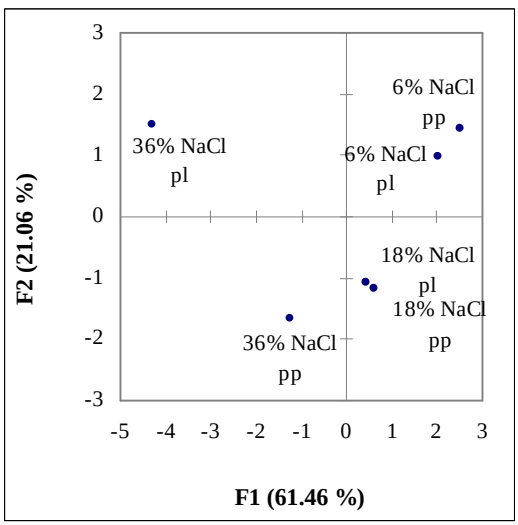

Fig. 2. PCA plot of treatments in reference to WHC and LF-NMR variables (figure 1); pl: parallel fibre direction; pp: perpendicular fibre direction

The high correlations between T_{21} ms and T_{21} area with WHC suggest that maximising intra-cellular water entrapment is key in improving WHC and in this study it can be seen that 6% NaCl was the most favourable concentration for this (Fig. 1 and Fig. 2).

T_{22} ms was increased by 36% NaCl ($p<0.05$). It may be that 6% and 18% NaCl caused extra-cellular water to become more bound, while 36% NaCl, the concentration causing the highest osmotic stress, removed this most loosely bound fraction. The curve width of this component was also increased by 6% NaCl ($p<0.05$). This may be attributed to the swollen 'soup-like' matrix induced by curing [4]. The area under the curve of T_{22} was not significantly affected by NaCl concentration, however it was affected by fibre direction ($p<0.05$). The findings suggested that curing the sample with fibre direction parallel to the axis of the cylinder, resulted in higher extra-cellular water entrapment.

As the NaCl concentrations used in this study are known to have contrasting effects on intra-/extra-cellular water entrapment, the findings support this theory of water compartmentalisation in meat and the strong correlations between WHC and NMR variables suggest the method is a successful indicator of meat-water interactions.

B. Diffusion coefficients

The sample cured with 6% NaCl slowly gained moisture at a rate of 2.74×10^{-9} m^2/s. Samples cured at higher concentrations became dehydrated at rates of 1.12×10^{-8} m^2/s and 1.93×10^{-8} m^2/s with 18% and 36% NaCl, respectively. This evidence that 6% NaCl results in a slow gain of moisture and that higher NaCl concentrations result in moisture loss supports the LF-NMR findings.

C. WHC

When comparing treated samples to the non-treated control, it was noted that 6% NaCl caused an increase in WHC above that of raw meat, while 18 and 36% NaCl caused a decrease in the WHC. These findings support the hypothesis that 6% NaCl caused increased intra-cellular water which is more tightly bound than extra-cellular. There was also a significant interaction with an increase in WHC at 36% NaCl with perpendicular fibre direction. The decrease in WHC of the parallel sample is most likely due to increased extra-cellular water which was indicated by the increased T_{22} area in LF-NMR findings.

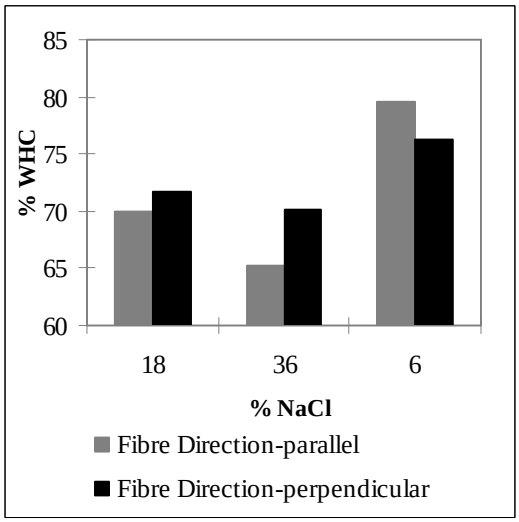

Fig. 3. Water-holding capacity of samples

D. Histology

Microscopy images showed visible swelling of fibres with 6% NaCl. 18% NaCl caused large extra-fibrillar spacing, while 36% NaCl produced changes consistent with a dehydrated structure.

IV. CONCLUSIONS

Firstly, these findings suggest that LF-NMR relaxation behaviour in meat is due to water compartmentalisation within macromolecules, intracellular and extracellular compartments and therefore, LF-NMR is a technique which allows for further understanding of water mobility and distribution in meat. Secondly, the results indicate that optimising intra-cellular water and minimising extra-cellular water is key in increasing WHC. The optimum NaCl concentration for this was 6% and was independent of fibre direction. However, as there was an indication that fibre direction does significantly affect WHC when curing with 36% NaCl, curing at a perpendicular fibre direction when working with high NaCl concentrations may result in enhanced WHC.

V. ACKNOWLEGMENTS

The authors wish to thank the Department of Agriculture, Fisheries and Food for their financial support through the Food Research Institutional Measure (FIRM).

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