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The effect of salt and fibre direction on water dynamics, distribution and mobility in pork muscle: A low field NMR study

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ABSTRACT

The effect of salt concentration and fibre orientation on water within the meat matrix was investigated by low-field nuclear magnetic resonance (LF-NMR), water-binding capacity (WBC), diffusion studies and histological analysis. Pork *M. longissimus thoracis et lumborum* samples were cured with 5.7, 15.3 or 26.3% w/w NaCl at a parallel or perpendicular fibre direction. NMR transverse (T_2) relaxation identified three water components (T_{2b}, T_{21} and T_{22}) which all exhibited characteristics correlated to WBC. Results indicated that T_{2b} increases with increasing NaCl concentration. Increasing intra-myofibrillar water and decreasing extra-myofibrillar water resulted in the highest WBC. Water diffused more quickly into the extra-myofibrillar space in samples cured at a parallel fibre direction. This water remained loosely bound in samples cured with the saturated solution (26.3% w/w NaCl) leading to decreased WBC. This study provides further information on water binding within the meat matrix by applying the results of LF-NMR to traditional water-binding theories.

1. Introduction

Curing is an ancient food preservation technique which can be dated back to 3000 BC (Shahidi & Samaranayaka, 2004). Traditional curing involved the use of high NaCl concentrations for preservation but as refrigeration became readily available, curing techniques evolved and now have the added aim of enhanced juiciness, tenderness and flavour (Feiner, 2006). Curing techniques are generally classified as wet or dry-curing. Wet curing is commonly performed by injecting a brine solution into the meat; however some traditional premium wet-cured meats are cured by immersion in a brine solution. Brine commonly contains water, sodium chloride, phosphates, sodium nitrite and other non-meat ingredients (Shahidi & Samaranayaka, 2004; Youling, 2005). Nitrite induces a desirable pink colour while functioning as a preservative (Shahidi & Samaranayaka, 2004). A product is labelled as cured if it contains nitrite; otherwise the product is labelled as 'uncured' (Krause, Sebranek, Rust, & Mendonca, 2011). Of all the brine ingredients, NaCl is the most important (Gou, Comaposada, & Arnau, 2003). The NaCl concentration in the final product ranges from 1 to 2.8% (Pegg, 2004). The level of NaCl in brine for injection-curing will depend on the injection level; the NaCl concentration in the brine will decrease as the injection level increases (Feiner, 2006). At low injection levels (10–30% weight gain), the concentration of NaCl in the brine will be high; commonly 18.5% w/w NaCl (Pegg, 2004). Meat products cured by traditional techniques, such as Wiltshire ham, are cured by immersion in almost saturated brine (20–24% w/w NaCl) (Feiner, 2006). Along with the anti-microbial effect of NaCl (Graiver, Pinotti, Califano, & Zaritzky, 2009) it acts as a structure-breaker allowing the filament lattice to expand and entrap water. This occurs due to electrostatic repulsion within the myosin filament (Offer & Knight, 1988). This expansion will expose protein side-chains for water binding (Feiner, 2006). Although the NaCl concentration in the final product is about 2% NaCl, prior to equalisation some proteins are exposed to the high levels of NaCl present in the brine. Myofibrils will swell to a maximum at 5.8% NaCl, after which protein denaturation and shrinkage will occur (Knight & Parsons, 1988) resulting in decreased water-holding capacity (WHC) (Feiner, 2006).

The WHC of meat can be defined as its ability to retain its own and added water (Hamm, 1961) and this has a direct influence on its juiciness, tenderness, and yield, thereby influencing its economic value (Huff-Lonergan & Lonergan, 2005). Due to its importance, research has been conducted on improving (Bertram, Meyer, Zhiyun, Zhou, & Andersen, 2008; Young, Karlsson, & Henckel, 2004) and better understanding WHC (Huff-Lonergan & Lonergan, 2005; Pearce, Rosenvold, Andersen, & Hopkins, 2011; Puolanne & Halonen, 2010; Youling, 2005). Although much information has been gained, there is still need for more knowledge on the foundations of bulk water-holding within meat (Puolanne & Halonen, 2010) and the effects of the addition of NaCl on water distribution and mobility within the filament lattice (Bertram, Karlsson, et al., 2001; Hansen, Van Der Berg, Ringgaard, Stødkilde-Jørgensen, & Karlsson, 2008).

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In recent years, low-field nuclear magnetic resonance (LF-NMR) has been employed to understand water mobility and distribution within meat (Bertram & Andersen, 2004). It has been described as an excellent indicator of myofibrillar geometry upon curing (Andersen, Andersen, & Bertram, 2007) and relaxation data has been correlated with WHC (Bertram, Andersen, & Karlsson, 2001; Brøndum et al., 2000; Renou, Monin, & Sellier, 1985). It has been suggested as a tool for fast non-invasive analysis of WHC in industry (Bertram & Andersen, 2004). NMR is performed by applying a radio frequency pulse to a sample within a magnetic field and assessing the relaxation properties of the proton nuclei of the sample (Charlton, 2009). By application of multi-exponential fitting of data, different water components may be identified as intra-myofibrillar or extra-myofibrillar (Bertram, Purslow, & Andersen, 2002). The relaxation time acts as an indicator for water mobility, while the area under curve can indicate the amount of water within the component (Bertram & Andersen, 2007; Bertram, Karlsson, et al., 2001; Pearce et al., 2011). Due to the extensive information gained through this technique, it has been used in meat science to assess water–protein interactions in pork treated with 0.9–9% w/w NaCl (Andersen et al., 2007), 3–5% w/w NaCl (Bertram et al., 2008) and 10% w/w NaCl (Bertram, Holdsworth, Whittaker, & Andersen, 2005) and fish treated with 15 or 25% w/w NaCl (Aursand, Gallart-Jornet, Erikson, Axelson, & Rustad, 2008). These studies have provided excellent information on changes in water–protein interactions as affected by NaCl however, a study assessing a wider range of NaCl concentrations would be beneficial in understanding the mechanistic actions of water-binding in meat. Also, the use of LF-NMR could help in further understanding results of previous studies which showed maximum meat hydration with NaCl at an ionic strength of 0.8–1.0 (Hamm, 1961) or 1M (Knight & Parsons, 1988).

Water dynamics also vary according to the NaCl concentration used (Foucat, Benderbous, Bielicki, Zanca, & Renou, 1995). Mathematical models have been designed to quantify the speed at which the brine diffuses into the meat (Graiver et al., 2009). Diffusion coefficients have been calculated for different brine concentrations in pork (Gou et al., 2003; Graiver, Pinotti, Califano, & Zaritzky, 2006), for different cure methods (Sabadini, Carvalho, Sobral, & Hubinger, 1998) and in muscles of varying connective tissue content (Hansen et al., 2008; Wood, 1966). Vestergaard (2004) studied the use of non-invasive techniques to determine diffusion coefficients and brine distribution in meat. Although this has led to an understanding of the movement of brine in meat, an added knowledge of the distribution and location of the water once diffused in the meat matrix could also be beneficial. This study aims to provide further information on water migration and water-binding within the meat matrix upon salting by assessing the effect of fibre direction and a wide range of NaCl concentrations.

2. Materials and methods

2.1. Meat sampling and treatment

Pork *M. longissimus thoracis et lumborum* (LTL) muscles of pH 5.5–5.8 were obtained at 72 h post-mortem from a commercial slaughterhouse. Cylindrical samples (35ϕ × 25 mm, 25 ± 0.5 g) were cored to have a fibre direction parallel or perpendicular to the main axis of the cylinder. All visible connective tissue was removed from the muscle and samples were generated from the lean tissue. Duplicate treatments were generated per muscle and location within the muscle was randomised with respect to treatment. A new muscle was used for each repetition of the experiment. The samples were inserted into polyethylene tubes (34ϕ × 130 mm) and the base was closed with a rubber stopper (Fig. 1). A brine solution (65 ml) containing 5.7, 15.3 or 26.3% w/w NaCl was placed 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. Three repetitions of the experiment were conducted.

2.2. Physico-chemical analysis

The pH of each muscle was taken prior to processing by direct insertion of a glass pH electrode EC-2010-11 (Refex sensors Ltd., Westport, Co. Mayo, Ireland) into the meat. The electrode was calibrated using pH 4 and pH 7 buffers at 25 °C and thermostated at 4 °C prior to the analysis. The result was taken as the average of three repetitions along the length of the muscle. Moisture content was determined by weight loss after overnight oven drying at 103 ± 2 °C (A.O.A.C., 1990). NaCl content was determined by standard titrimetric Volhard method (Kirk & Sawyer, 1991).

2.3. NMR measurement

The top 10 g of the cured sample were placed in sealed NMR tubes (15 mm diameter) and held at 25 °C in a water bath (Model No. GD100, Grant Instruments Ltd., Barrington, Cambridge CB2 5QZ, England) for 1 h. The LF-NMR data was generated using a Maran Ultra NMR spectrometer (Oxford Instruments Molecular Biotools Ltd., Abingdon, UK) 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 (Carr & Purcell, 1954; Meiboom & Gill, 1958). Measurements were obtained using a τ value of 150 μs and a relaxation delay of 5 s. Each measurement was the result of 16 scan repetitions. Three measurements were carried out on each sample.

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). Mean relaxation times, curve widths (CW) and area under the curve values were determined using Sigmaplot software (Version 11, Systat Inc. USA).

2.4. WBC

Following NMR analysis, WBC was determined on samples. Analyses were performed in duplicate. Samples were weighed (5 g) and placed into specially designed centrifuge tubes as described by Farag, Duggan, Morgan, Cronin, and Lyng (2009). Cooking (90 °C, 10 min) and centrifugation (220 g, 10 min, 4 °C) were performed as described by Hayes, Desmond, Troy, Buckley, and Mehra (2005). The WBC was calculated as the weight change before cooking and after centrifugation.

2.5. Diffusion coefficients

Diffusion coefficients were calculated with a separate experiment. Samples were cored and placed in a tube with parallel or perpendicular fibre direction as previously described but the meat cylinder length was

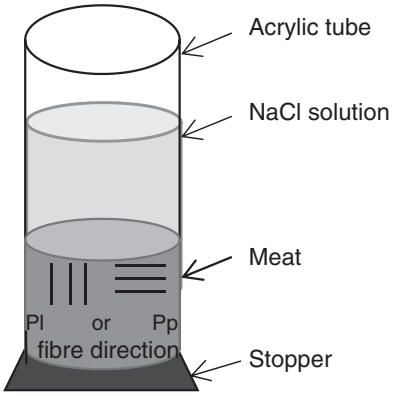

Fig. 1. Experimental set-up: meat curing vessel where Pl refers to a parallel fibre direction and Pp refers to a perpendicular fibre direction.

increased to 50 mm. Samples were cured for 2 h at 4 °C on a platform shaker (Stuart SSL2, Bibby Scientific Limited, Staffordshire, UK) at 200 cycles per minute to ensure constant agitation of the brine. Following treatment, the brine was discarded and the sample was rinsed with deionised water and blotted dry. The sample was then stored at −18 °C inside the salting vessel for 2 h to give rigidity and then sliced using a slicer (800 S, Avery Berkel, England, UK) to generate 11 × 2 mm slices. Slices were analysed for moisture and NaCl content. Diffusion coefficients for water (D_w) and NaCl (D_s) were calculated with Eqs. (1) and (2) according to Fick's second law as modified by Luna and Chavez (1992) and Pajonk, Saurel, and Andrieu (2003), respectively.

$$\frac{C_o - C(x,t)}{C_o - C_w} = 1 - \mathrm{erf}\left(\frac{x}{2\sqrt{D_w.t}}\right) \quad (1)$$

$$\frac{C_s - C(x,t)}{C_s - C_0} = \mathrm{erf}\left(\frac{x}{2\sqrt{D_s.t}}\right) \quad (2)$$

Where, C represents the instantaneous NaCl/water concentration in the meat, C_w is the water content at the surface of the meat sample after brining, C_s is the NaCl content of the brine and C_0 is the initial NaCl/water content of the meat sample, x is the distance from the meat surface (m) and t is time (s), while D_w and D_s are the diffusion coefficients (m^2/s) for water and salt, respectively.

2.6. Histology

Pork samples (2 × 1.5 × 1 cm) were cured for 22 h by immersion in a brine solution of 5.7, 15.3 or 26.3% NaCl. Post-salting, samples were rinsed with deionised water and blotted dry. A non-treated sample acted as a control. Samples were immediately fixed in a 10% formaldehyde solution (Fisher Scientific, Dublin 15, Ireland) for 24 h and then processed to have transverse fibre direction as described by Shirsat, Lyng, Brunton, and McKenna (2004).

A light microscope (Model No. 523155, Labophot-2, Nikon, Japan) was used to examine the effect of NaCl concentration on transverse fibres. Images were recorded using a digital video camera (QImaging micropublisher 3.3 RTV, Canada). Three slides of each treatment were generated. Ten sites were selected at random from these slides. Each site contained an average of 480 fibres. Images were analysed using Image-pro plus software (Version 6.2.1, Media Cybernetics, USA). Images were analysed for the area and diameter of each fibre.

2.7. Statistical analysis

Two separate forms of data analysis were performed. Firstly, all data underwent a two-way analysis of variance (ANOVA) with factors of NaCl concentration and fibre direction (GenStat, 12th Edition, VSN International, UK). Secondly, principal component analysis (XLSTAT version 2011.2 Addinsoft) was performed for all NMR variables and WBC with the treatments of NaCl concentration and fibre direction. A separate PCA was performed in order to calculate correlations between all histological measurements and NMR as these help in understanding the distribution of the water within the meat matrix upon swelling. In order to find these correlations a non-treated control was included and therefore, the effect of fibre direction was removed. PCA is a modern exploratory data analysis tool, which finds the directions of the data in a multidimensional space and in turn, summarises the data allowing for identification of correlations between samples and treatments (Hossain, Patras, Barry-Ryan, Martin-Diana, & Brunton, 2011). Application of PCA to NMR relaxation data has previously been shown to give beneficial data enhancement and reduction (Bechmann, Pedersen, Nørgaard, & Engelsen, 1999).

3. Results

3.1. NMR

A representative distribution of NMR T_2 measurements after multi-exponential fitting can be seen in Fig. 2. Three peaks were identified as T_{2b}, a fast minor component (2–5% of total water) with a relaxation time of 7–13 ms, T_{21}, a major component (86–92% of total water) with a relaxation time of 39–54 ms and T_{22}, a slow component (5–9% of total water) with a relaxation time of 133–177 ms. Table 1 displays the least-squared means of relaxation times, curve widths (CW) and areas of each component. The relaxation times of all three components were significantly affected ($p < 0.05$) by NaCl concentration. There was an increase in T_{2b} ($p < 0.001$) with increasing NaCl concentration but the area of the component or the curve width did not change ($p > 0.05$). The opposite was observed for T_{21}, whereby relaxation time decreased with increasing NaCl concentration ($p < 0.001$) resulting in 5.7% w/w NaCl causing the highest T_{21} relaxation time. The same trend ($p < 0.001$) was observed for the area of the T_{21} component whereby 5.7% w/w NaCl caused the area of this component to be at its largest. All T_{22} characteristics i.e. relaxation times, curve widths and area were affected by NaCl concentration or fibre direction. The relaxation time of the T_{22} component was increased ($p < 0.05$) by 26.3% w/w NaCl, while the curve width of the component broadened when meat was cured with 5.7% w/w NaCl. The area of this component was larger ($p < 0.05$) in samples cured at a parallel fibre direction. There was no significant interaction between NaCl concentration and fibre direction on NMR parameters.

3.2. WBC

The WBC of cured pork decreased with increasing NaCl concentration ($p < 0.001$). An effect of fibre direction was found in the WBC results (Fig. 3) whereby the WBC decreased ($p < 0.05$) in samples cured at a parallel fibre direction for 26.3% w/w NaCl only. This was also detected in the PCA score plots (Fig. 4A and B) of distributed T_2 variables and WBC, where F1 and F2 explain 61.5 and 21.1% of the variation, respectively. Significant correlations were found between relaxation times of all three NMR components and WBC (Table 2). These indicate that as WBC decreased, the relaxation time of both T_{2b} and T_{22} increased, while relaxation time of T_{21} decreased. WBC also had a positive correlation with T_{21} area and negative correlation with T_{22} area. This combination was achieved with 5.7% w/w NaCl independent of fibre direction.

3.3. Diffusion coefficients

Water and NaCl concentration gradients at the saline/meat interface varied according to the NaCl treatment applied. In all treatments NaCl migrated into the meat (Fig. 5A). However, water content of the

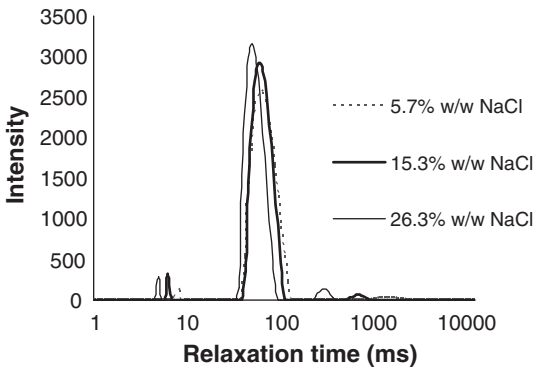

Fig. 2. Typical distribution of multi-exponentially fitted transverse relaxation (T_2) data of samples cured with varying NaCl concentration.

Table 1
Least-square means, standard errors and *p*-values for LF-NMR parameters (T_{2b}, T_{21} and T_{22}) as affected by brine concentration and fibre direction*.

	NaCl concentration (% w/w)			Fibre Direction		Standard error	*p*-value NaCl	*p*-value fibre direction
	5.7	15.3	26.3	Parallel	Perpendicular			
T_{2b} (ms)	6.4[a]	9.6[b]	12.3[c]	9.5	12.3	±2.8	0.001	NS***
T_{21} (ms)	53.9[a]	49[b]	40[c]	48.1	47.1	±2.9	0.01	NS
T_{22} (ms)	145.1[a]	140.2[a]	167.6[b]	115.4	146.5	±20.7	0.05	NS
T_{2b} CW**	1.2	1.1	1.3	1.2	1.1	±0.3	NS	NS
T_{21} CW	64.9	65	58.4	58.4	67.1	±22.3	NS	NS
T_{22} CW	223[a]	155[b]	147[b]	171	179	±63.1	0.05	NS
T_{2b} area	88.2	64.4	105.3	88.5	83.5	±39.9	NS	NS
T_{21} area	102147[a]	85957[b]	60399[c]	82035	83634	±7331.9	0.001	NS
T_{22} area	8410	12509	12646	13242[a]	9135[b]	±5314.9	NS	0.05

* Different superscript letters (a–c) across the row indicate significant differences treatments.
** CW indicates curve width.
*** NS indicates non-significant.

meat was increased by 5.7% w/w NaCl, where as the 15.3 and 26.3% w/w NaCl treatments caused water to migrate out of the meat (Fig. 5B). Values of the diffusion coefficient ranged from 0.8 to 6.8 × 10^{-10} m^2/s and 2.1 to 2.4 × 10^{-10} m^2/s for water and NaCl, respectively (Table 3). While NaCl diffusivities (D_s) did not differ between NaCl treatments, water diffusivity (D_w) was lower for the 5.7% w/w NaCl treatment. Overall water and NaCl diffusivities were slightly higher in samples cured at a parallel fibre direction.

3.4. Histology

Analysis of images (Fig. 6) showed that all NaCl concentrations caused swelling of the fibres ($p < 0.001$) in comparison to the non-treated control. The largest overall fibre area and diameter were found in the sample cured with 5.7% w/w NaCl. This was significantly larger than the swelling observed with 26.3% w/w NaCl (Table 4). Samples treated with 15.3% w/w NaCl did not differ in any properties from samples treated with 5.7% and 26.3% w/w NaCl. Correlations were found between some LF-NMR variables and histological variables. It can be seen that as the overall size of the fibres increased, the T_{21} relaxation time increased. The T_{22} area decreased as the overall fibre size increased.

4. Discussion

Meat curing is commonly used to increase shelf-life and improve juiciness, flavour and texture. The most important functional ingredient to induce such changes is NaCl. Two mechanisms have been proposed for NaCl causing structural changes within the meat matrix which allow for water uptake. Firstly, negatively charged ions cause

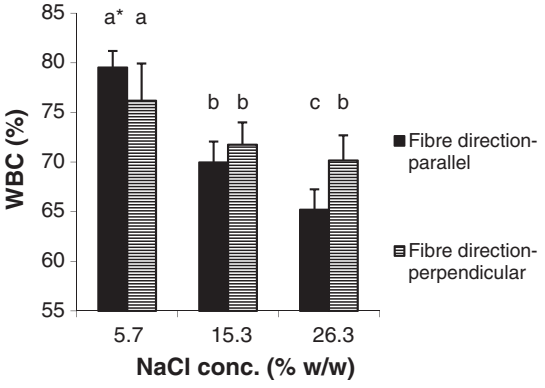

Fig. 3. Least-Square Means of water-binding capacity for samples cured with difference NaCl concentrations at different fibre orientations; error bars indicate the standard deviation between three replicates. *Different letters (a–c) indicate significant differences ($p < 0.05$) between treatments.

increased electrostatic repulsion between filaments (Hamm, 1972) and secondly, depolymerisation of the thick filament within the myofibril removes constraint allowing for swelling (Offer & Trinick, 1983). However, the degree of lateral expansion is dependent on NaCl concentration (Knight & Parsons, 1988). Further knowledge about water within the filament lattice and WBC as affected by salt may help in further understanding the mechanisms behind bulk water holding in the meat matrix.

In this study three peaks were identified through multi-exponential fitting of T_2 relaxation data. Characteristics of the peaks were similar to findings reported by Bertram, Karlsson, et al., 2001 and Shaarani, Nott, and Hall (2006). The peaks are thought to be directly related to three water components in muscle tissue (Bertram & Andersen, 2007; Pearce et al., 2011). The first component, bound water (T_{2b}), is water tightly bound to charged muscle proteins (7–13 ms). The second component is immobilised water (T_{21}) which is located in the protein-dense myofibrillar network, within the space between the thick and thin filaments (intra-myofibrillar water; 39–54 ms). The final component is free water (T_{22}), containing sarcoplasmic proteins which, is loosely held between fibres (extra-myofibrillar water; 133–177 ms) (Bertram, Karlsson, et al., 2001; Huff-Lonergan & Lonergan, 2005; Pearce et al., 2011).

It has been suggested that water very tightly bound to macromolecules (T_{2b}) is independent of any mechanical stress and micro- or macro-structural changes in the meat matrix (Bertram, Karlsson, et al., 2001; Pearce et al., 2011). However, the findings of this study show changes in the relaxation time of T_{2b} due to NaCl concentration (Table 1). When Bertram, Karlsson, et al., 2001 adjusted the electrostatic interactions in meat using NaCl, they found changes in T_{21} and T_{22} components but none to T_{2b}. They did however, compare a non-treated control to 5% NaCl treated meat and did suggest that further studies of the effect of NaCl on water distribution in meat were needed. Likewise, Andersen et al. (2007) treated meat using a NaCl range of 0–9% and reported no changes to the relaxation of the T_{2b} component. The range of NaCl treatments used in this study (5.7–26.3% NaCl) was such that changes were detected. At 5.8% w/w NaCl, the filament lattice expands to a maximum, increasing the surface area within the myofibril and therefore exposing a greater number of macromolecules as sites for water binding (Bertram et al., 2008). Side chains of proteins available for water binding include carboxyl-, amino-, hydroxyl- and sulfhydryl-groups while the carbonyl- and imido-groups of the peptide bonds may also play a role (Hamm, 1961). The lowest T_{2b} relaxation corresponds to the availability of protein side-chains due to myofibrillar swelling (Table 1). Knight and Parsons (1988) proposed that high NaCl concentrations may have a destabilising effect on the α-helical structure of the myosin tail causing it to fold and produce smaller structures and/or the lack of swelling is due to myosin being salted out. Water was removed from samples treated with 15.3 and 26.3% w/w NaCl (Fig. 5B), meaning that some sarcoplasmic and myofibrillar

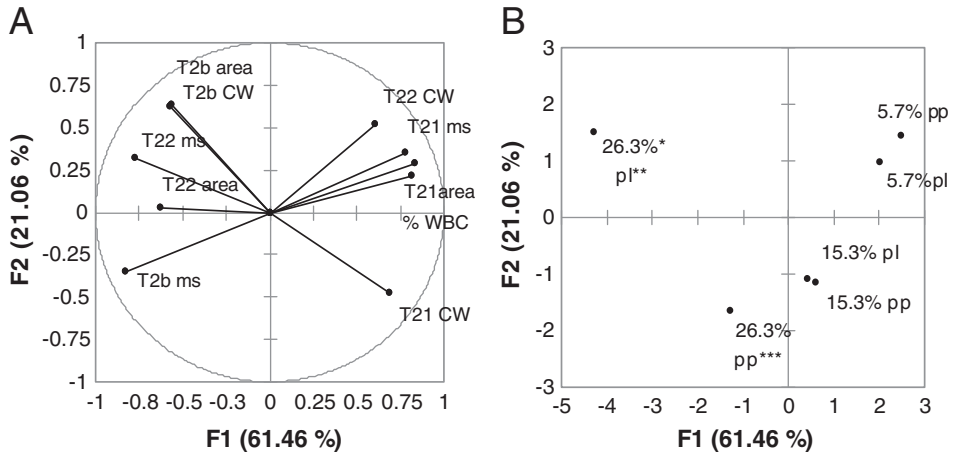

Fig. 4. PCA vector plot of WBC and LF-NMR variables (A); PCA score plot of treatments with WBC and LF-NMR variables (B). * NaCl concentrations (% w/w). ** pl: parallel fibre direction. ***pp: perpendicular fibre direction.

proteins could have been removed from the meat. This would reduce the amount of protein side-chains for water-binding. Additionally, high NaCl concentrations will result in protein denaturation, further reducing the number of macromolecules available as water-binding sites. The increase in T_{2b} with increasing NaCl concentration suggests that as the proteins became denatured, less hydrophilic groups were available for water-binding. This was also evident in the negative correlation between T_{2b} relaxation and WBC (Table 2).

In this study, 5.7% w/w NaCl caused the highest T_{21} relaxation time and T_{21} area. Although the water is less bound compared to the 15.3 and 26.3% w/w NaCl treatments, there is more water contained within this component, reflected by the increased T_{21} area (Table 1). This increased immobilised intra-myofibrillar water resulted in increased WBC (Fig. 3) and this was reflected in the correlation between the two (Table 2). Salting of pork with 5.7% w/w NaCl has been reported several times to cause the highest amount of swelling and therefore intra-myofibrillar water (Andersen et al., 2007; Gou et al., 2003; Knight & Parsons, 1988). The relaxation time of T_{21} can act as an indicator of myofibrillar geometry (Brøndum et al., 2000) as it detects the increased distance between the thick and thin filaments for the water molecules to move (Bertram, Kristensen, & Andersen, 2004). This has been demonstrated by increased T_{21} relaxation induced by swelling upon salting (Bertram, Karlsson, et al., 2001; Bertram et al., 2004; Bertram et al., 2008) and also decreased T_{21} relaxation induced by lateral shrinkage of myofibrils upon cooking (Shaarani et al., 2006). This swelling due to 5.7% w/w NaCl was also reflected by an increase ($p < 0.05$) in the T_{22} curve width which indicates a loss of homogeneity. Andersen et al. (2007) described broadening of a peak upon salting as the formation of a 'soup-like' matrix where intra- and extra-myofibrillar spaces become difficult to distinguish from one another.

A large decrease in intra-myofibrillar water (T_{21} area) was evident when the meat was treated with 26.3% w/w NaCl. As previously discussed, high NaCl concentrations will result in protein denaturation and 'salting-out' (Knight & Parsons, 1988). Graiver et al. (2006) reported reduced WHC above 200 g/l NaCl and water losses above 330 g/l NaCl. As 26.3% w/w NaCl caused the intra-myofibrillar water to decrease, it may be that the osmotic stress caused myofibrils to expel water into the extra-myofibrillar space where it was less bound (Knight & Parsons, 1988) and this was detected by an increase in the relaxation time of the extra-myofibrillar water (T_{22}).

The T_{22} area was not affected by NaCl concentration, however samples cured at parallel fibre direction had increased ($p < 0.05$) extra-myofibrillar water. To the best knowledge of the authors, LF-NMR has not been used to assess the effect of salting meat at a particular fibre direction. For that reason, the result may be better understood when referring to the diffusion coefficient results (Table 3). These results show

Table 2
Correlations of WBC with LF-NMR variables.

Variable	Significance	Correlation
T_{2b} ms	$p \leq 0.001$	−0.86
T_{21} ms	$p \leq 0.001$	0.79
T_{22} ms	$p \leq 0.05$	−0.67
T_{2b} area		−0.34
T_{21} area	$p \leq 0.001$	0.87
T_{22} area	$p \leq 0.05$	−0.79
T_{2b} CW*		−0.36
T_{21} CW		0.54
T_{22} CW	$p \leq 0.05$	0.64

* CW indicates curve width.

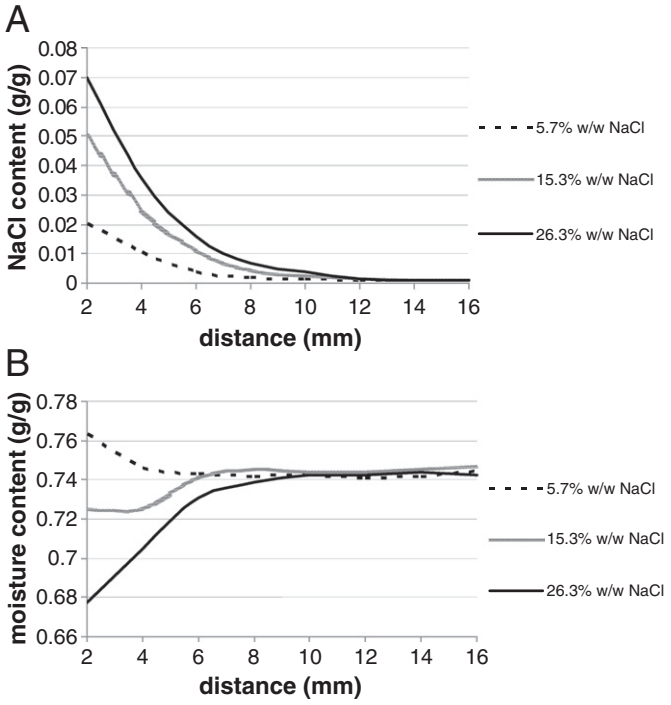

Fig. 5. NaCl (A) and moisture (B) migration in meat samples treated with different NaCl concentrations.

Table 3
Water diffusivities (D_w) and NaCl diffusivities (D_s) as affected by fibre direction and NaCl concentration.

	$D_w \times 10^{-10}$ (m^2/s)	$D_s \times 10^{-10}$ (m^2/s)
5.7% NaCl pl*	0.84	2.38
15.3% NaCl pl	6.78	2.38
26.3% NaCl pl	5.02	2.38
5.7% NaCl pp**	2.06	2.12
15.3% NaCl pp	5.25	2.14
26.3% NaCl pp	4.47	2.1

* pl: parallel fibre direction;
** pp: perpendicular fibre direction.

Table 4
Least-square means of histological variables as affected by NaCl concentration and correlated LF-NMR variables of values greater than ±0.7*.

	Treatments (NaCl conc. % w/w)				Correlated variable	
	0	5.7	15.3	26.3	T_{21} ms	T_{22} area
Area (μm)	1635.7^{c}	2478.1^{a}	2185.4^{ab}	2006.9^{bc}	0.77	−0.98
Diameter (μm)	56.4^{c}	70.1^{a}	65.3^{ab}	62.5^{bc}	0.77	−0.98

* Different superscript letters (a–c) across the row indicate significant differences ($p < 0.001$) between treatments.

slightly higher NaCl and water diffusion at a parallel fibre direction for 15.3 and 26.3% w/w NaCl treated samples. Zhang, Xiong, Liu, Xu, and Zhao (2011) revealed that diffusion anisotropy exists when NaCl migrates into meat. Dwyer, Allen, and Buckin (2000) found anisotropy due to fibre direction for the velocity and attenuation of ultrasonic waves in meat. Many studies have found heat and water transfers to be faster in meat at a parallel fibre direction, rather than perpendicular (Foucat et al., 1995; Godsalve, Davis, & Gordon, 1977; Gou, Comaposada, & Arnau, 2002; Thorvaldsson & Skjöldebrand, 1996) and it has been attributed to a shorter path for molecules to travel at parallel fibre direction (Godsalve et al., 1977; Thorvaldsson & Skjöldebrand, 1996). That is, at a perpendicular fibre direction, molecules will have to penetrate the selectively permeable fibre membranes or alternatively move around fibres, resulting in slower diffusion. There is also free water in the endomysium and perimysium to aid faster extra-fibrillar diffusion. A decrease in D_w was observed when samples were cured with 5.7% w/w NaCl. As this NaCl concentration induced swelling of the fibres, an increased resistance to mass transfer may have developed (Zhang et al., 2011) while 26.3% w/w led to the greatest fibre shrinkage and subsequent extra-fibrillar spacing for diffusion and extra-myofibrillar water (T_{22} area). Graiver et al. (2006) also found an increase in salt diffusivity with increasing NaCl concentration owing it to the disrupted meat matrix at high NaCl concentrations.

The calculated values of the NaCl diffusion coefficient of 2.10–2.38 × 10^{-10} m^2/s compare very favourably to those seen in the literature. Fox (1980) obtained a value of 2.2 × 10^{-10} m^2/s at 5 °C in *L.dorsi* (LTL) pork tissue when an 18% NaCl brine was applied. The same value was also found by Wood (1966) and Gros, Dussap, and González-Méndez (1984) at 12 and 2 °C, respectively. Sabadini et al. (1998) observed a diffusion coefficient of 2.3 × 10^{-10} m^2/s for saturated NaCl brine in beef at 10 °C. It was found that the NaCl diffusion coefficient (D_s) was unaffected by brine concentration. This behaviour was also observed by Fox (1980) and Wood (1966) for pork tissue and Sarang and Sastry (2007) for vegetable tissue. However, it must be noted that meat is a complex material prone to changes in geometry and protein content upon salting. For this reason Fick's 2nd law does not describe the diffusion in meat perfectly. Also, meat is permeable to ions so osmosis is another form of mass transfer which can occur (Thorarinsdottir, Arason, Thorkelsson, Sigurgisladottir, & Tornberg, 2010).

Analysis of the histological images (Fig. 6) along with relationships to NMR variables (Table 4) further enhances the understanding of water distribution within the meat matrix as affected by NaCl concentration. The largest amount of swelling was caused by 5.7% w/w NaCl; however swelling was evident with all NaCl treatments. This is similar

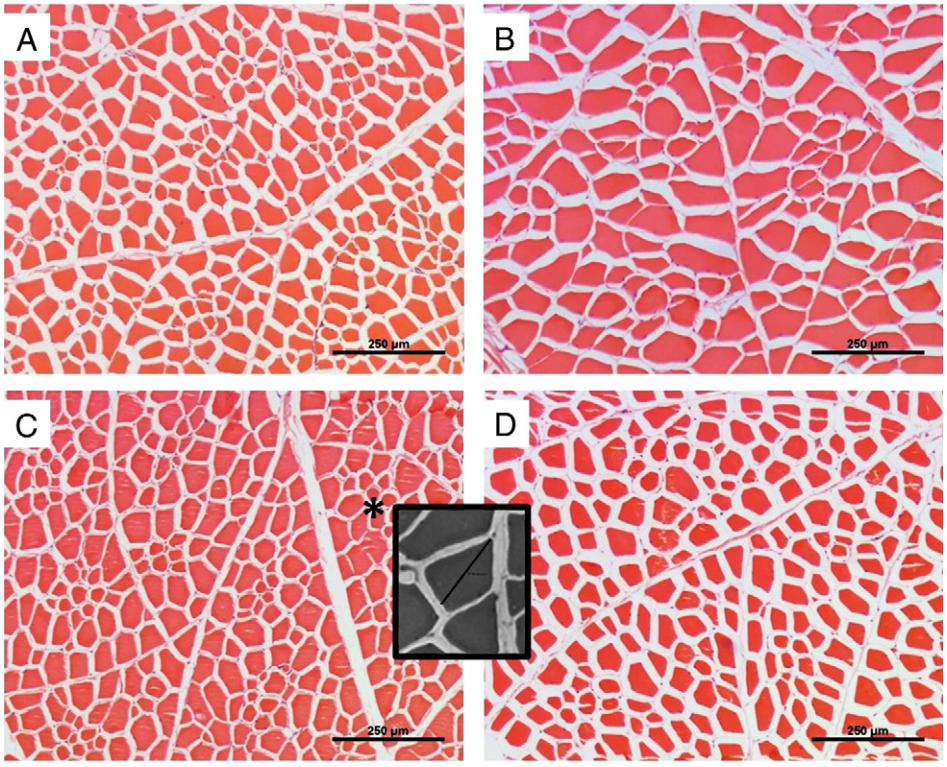

Fig. 6. Light microscope images of transverse meat sections cured with different NaCl concentrations: control (A), 5.7% w/w NaCl (B), 15.3% w/w NaCl (C), 26.3% w/w NaCl (D).
* Diameter was measured as the length of the longest line joining two points of object's outline and passing through the centroid.

to the findings of Knight and Parsons (1988) who reported that varying degrees of myofibrillar swelling will occur at all NaCl concentrations. A positive correlation between the area of the fibre and T_{21} relaxation was found ($r = 0.77$). This further supports the fact that T_{21} relaxation can act as an indicator of increased intra-myofibrillar water, as previously mentioned. This result along with the simultaneous decrease in T_{22} area ($r = -0.98$) as the fibres swell indicates that water does indeed become trapped within the myofibril during swelling and less water remains in the loosely bound extra-myofibrillar space. This combined effect results in the highest WBC (Bertram, Karlsson, et al., 2001). This supports the theory of Hamm (1961) which suggests that NaCl will increase the electrostatic repulsion within the myofibril through binding of Cl^- ions to carboxyl groups on the myosin molecule.

Good correlations of WBC with the relaxation times of all components, the size of the intra-myofibrillar water (T_{21} area) and the extra-myofibrillar water (T_{22} area) were evident. As outlined above, an increase in relaxation of the T_{2b} component may indicate 'salting-out' and protein denaturation. The positive correlation of WBC with T_{21} area along with the corresponding negative correlation with T_{22} area (Table 2) indicates that increasing intra-myofibrillar water and decreasing extra-myofibrillar water are key to maximum WBC. Renou et al. (1985) were first to describe correlations between NMR relaxation and WBC. Later, NMR was demonstrated to successfully indicate decreased WHC in pork (Andersen et al., 2007; Brøndum et al., 2000). It was first presumed that one component, T_{22}, was closely correlated with drip loss (Bertram, Dønstrup, Karlsson, & Andersen, 2002) but these results along with those of Bertram, Purslow, et al. (2002) prove that WHC is influenced by structural changes to the myofibril which leads to changes in water distribution inside and outside of the myofibril. Thus, characteristics of all three components within the relaxation curve should be taken into account when drawing conclusions on the WBC of meat. It is also interesting to note that these correlations were found between LF-NMR and WBC results taken on different matrices (raw vs. cooked), therefore the LF-NMR results of raw products may provide some prediction of the final WBC of cooked products.

An effect of fibre direction on WBC was evident in samples cured with 26.3% w/w NaCl only. It may be possible that 5.7 and 15.3% w/w NaCl caused sufficient fibre swelling to remove anisotropic effects but the 'salting-out' at 26.3% w/w NaCl resulted in very little swelling so anisotropy for diffusion remained. As extra-myofibrillar water is loosely held 'free water', this led to the 26.3% w/w NaCl sample having reduced WBC.

5. Conclusions

Findings of this study fit with traditional WBC theories; however LF-NMR provided further evidence and insight. All three LF-NMR water components indicate changes in the meat matrix, water distribution and subsequent water-binding. An increase in T_{2b} may be attributed to protein denaturation and loss of protein side-chains as sites for water-binding. In order to have maximum WBC, the aim must be to increase intra-myofibrillar water and decrease extra-myofibrillar water. This was best achieved by salting pork with 5.7% w/w NaCl independent of fibre direction. When salting with a saturated solution where little myofibrillar swelling occurs, there is faster diffusion at a parallel fibre direction resulting in increased extra-myofibrillar water and decreased WBC.

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