Original paper

The Distribution of Water in Pork Meat during Wet-curing as Studied by Low-field NMR

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The objective of the present work was to investigate water distribution in porcine muscle during wet-curing using both the low-field (LF) NMR T_2 components and traditional methods. Longissimus muscles were cut into 1 cm³ and immersed in brine solution (15% NaCl, w/w) at 4°C. The analysis of NMR and AOCO indicated that with increasing curing time there was greater uptake of water by the meat. Population of T_2 suggested that redistribution occurred between the protein-associated water, immobilized water and free water: the immobilized water increased while the others decreased. Aw and pH decreased during curing while WHC increased at first then kept decreasing after curing for 1h. DSC indicated that actin and myosin in meat were denatured. The conclusion is that with the swelling of meat structure by NaCl, water transferred from brine to meat surface firstly, then continued moving into the muscular tissue and converted to immobilized water mostly.

Keywords: water distribution, curing, LF-NMR, porcine

Introduction

Salting of meat is one of the most traditional methods for the preservation of meat and in recent years has become an important technology for the enhancement of meat quality, particularly those relating to sensory characteristics. There are many cured products in China and elsewhere such as water-boiled salted duck and dried ham, which are highly regarded for their delicate flavor and long shelf life. Salting process methods include wet-curing, dry-curing, injection salting or a combination of both. Since wet-curing retains more water in meat and gives higher weight yields, together with a shorter processing time, it has become popular as a pre-salting step prior to dry salting (Nguyen *et al.*, 2010).

Mass transfer of salt, water, protein and fat occurs during wet-curing between meat muscles and the brine (Hansen *et al.*, 2008;

Graiver *et al.*, 2009; Goli *et al.*, 2011). Apart from the transfer of salt, water transfer is one of the most important factors since it is the main constituent of meat and has a large impact on meat quality (Gianfrancescot *et al.*, 2012), and therefore is important to the meat industry. Research has shown that there are three different water components in meat. The first is protein-associated water where the charged hydrophilic groups on the muscle proteins tightly bind water; the second is immobilized water which accounts for up to 85% of the myowater and this is located within the thick filaments and between the thick and thin filaments of the myofibril and the last is free water, which is held only by capillary forces, and the orientation is independent of the charged group (Pearce *et al.*, 2011). During the curing process, beside of water transfer between meat and brine, exchange may occur among the three components

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of water in meat. The change of water state in the meat can affect the rate of curing, the shelf life texture and water holding capacity (WHC) of the meat product. In recent years, many studies have dealt with water distribution during curing, mainly on the diffusion kinetics and the parameters which affect the diffusion rate. Effects of salt concentration and temperature on the mass transfer kinetics have mainly been explored, however difficulties in identifying the three water compartments has made it difficult to predict water exchange. To our knowledge, only Loic Foucat (1995) and Ida G. Aursand (2010) have attempted to explain these changes in terms of the properties of water during curing meat using LF-NMR, MRI or MR microscopy.

Nuclear magnetic resonance (NMR) relaxation, which is an effective method to investigate the water content and its distribution in food, has been widely used in meat research (Andersen and Rinnan, 2002; Marcone *et al.*, 2013). Transverse relaxation time (T_2) has the advantages that it is sensitive to the existence of water in several phases, so it has been used widely to identify the state of water. In most LF-NMR analyses, T_2 values can be separated into three components, firstly a minor component between 1 and 10ms (T_{2b}) is referred to the protein-associated water, major component ranging from 40-60ms (T_{2i}) representing the immobilized water and lastly, a component between 100 – 250ms (T_{2z}) representing the extra-myofibrillar water (Bertram *et al.*, 2002; Li *et al.*, 2012). In addition, the population of T_2 is related to the water content obtained using the AOAC (Marcone *et al.*, 2013). Several studies have investigated the mechanism of water distribution during the cooking process of meat by the NMR technology (Bertram *et al.*, 2005; Mortensen *et al.*, 2006). Bertram and colleagues also investigated the WHC of meat using T_2 relaxation and they suggested that the area relating to the slowest population was correlated with the amount of expelled water. Additionally, NMR has been used to investigate the properties of water during frozen storage (Bertram *et al.*, 2007). However there have been few NMR investigations on water exchange during meat curing.

The objective of the present study was to identify the water distribution and its exchange in meat during curing by using LF-NMR to investigate 1) the mobility of water between the brine solution and meat, 2) the change of three water components in meat and 3) to explore the relationship between T_2 values and meat qualities such as WHC and water activity (Aw). Overall, the aim of these investigations is to develop a theory of water transfer and interactions in meat during the salting process to enable greater productivity and improved curing quality by controlling the salting process.

Materials and Methods

Raw material The posterior portions of three pork loins (*M. longissimus dorsi*) were purchased from local supermarket and stored at 4°C until required for trials (within 48 h). Edible salt

(NaCl) used for preparation of brine solutions was also obtained from a local supermarket.

Brining and sampling Pork loins were cut into 1 cm³ cubes and individually weighed (about 1.1 g). The edible salt was dissolved in distilled water (15%, w/w) for use as brine. The meat cubes were randomly allocated to salting treatments by immersing them in brine at a ratio of 1:3 (meat:brine, w/w). In order to prevent brine evaporation during treatment, the samples were kept in sealed plastic container. The salting process was carried out at 4°C for 5 h. To calculate the weight change during curing, samples (n = 3) were weighed at each sampling point (0, 1, 2, 3, 4 and 5 h) after draining for 1-2 min on a grid and then patted dry with absorbent paper reduce excess surface moisture. Then several cubes were chosen randomly to determine water content, Aw, water holding capacity and for DSC. Finally three meat cubes from each sampling time were immersed separately in 3 mL brine in three 15 mm tubes for NMR measurement. All determinations were carried in triplicate.

Water content and Aw measurement Moisture content was measured by drying 1 g of minced meat at 105°C for 15 h (AOAC, 1990). The water content was calculated as the percentage of water lost from the meat after drying. Aw was determined at 25°C at the surface of each minced sample using an activity meter instrument based on the dew-point method, having an absolute error within 0.003 (Lab Master aw, China).

Determination of WHC and pH WHC was described as pressurization loss in this paper and determined using the filter paper press method. The meat cubes were weighed (to within 0.001 g) and then pressed between 36 filter papers with 35 kg force for 5 min (YYW-2, China). It calculated as percentage of water lost from the meat after compression. Pressurization loss is on the contrary to WHC. The pH was measured with a pH-meter. The meat cubes were homogenized with distilled water at a ratio of 1:10. The mensuration was carried out in the homogenate.

DSC analysis DSC analyses were performed on 0.4 g sample cut from the center of each meat cubes and then encapsulated in an aluminum pan. Each sample was initially held at 20°C for 3 min and then heated to 100°C at a rate of 1°C min⁻¹ (TA MC-DSC America). Peak temperatures were obtained from the DSC thermogram.

NMR measurements A 1 × 1 × 1 cm³ meat cube was immersed in 3 mL brine and they were placed in a cylindrical glass tube (15 mm in diameter) at 4°C. This tube fitted into the NMR probe of diameter 18 mm. The NMR relaxation measurements were performed on a Niumag Benchtop Pulsed NMR Analyzer PQ001 (Niumag MicroMR, China) with a resonance frequency for protons of 22 MHz. Transverse relaxations (T_2) was measured using the Carr-Purcell-Meiboom-Gill sequence (CPMG). The T_2 measurements were performed with a τ -value (time between 90° pulse and 180° pulses) of 400 μ s. Data from 18000 echoes were acquired as 16 scan repetitions. The relaxation measurements were performed at 32°C. Post processing of NMR T_2 data distributed

exponential fitting of CPMG decay curves were performed by Multi-Exp Inv Analysis software (Niumag Electric Corp., Shanghai, China).

Statistical analysis Analysis of results was carried out using SPSS20.0, the significant differences between mean values of coefficient of variance were evaluated using one-way ANOVA. These variables were tested to the normalized Shapiro-Wilk normality test and to Bartlett's homogeneity test of variance. The Kruskal-Wallis test was used to test samples that did not fit the normal distribution. The correlation coefficients for the indexes were evaluated using correlation analysis. Differences between samples were considered to be significant at $p < 0.05$.

Results and Discussion

The findings in the present study are based on the use of a 15% brine solution for curing. Previous studies have reported that different salt concentrations may lead to different curing rates, yield weights and textures of the cured meat product (Deumier *et al.*, 1996; Graiver *et al.*, 2006; Gallart-Jornet L *et al.*, 2007). Higher brine concentrations usually induce higher rates of salt absorption, with less total weight, and water weight increase, which is beneficial for practical production. However, the changes in meat textural properties such as hardness and springiness as well as WHC caused by high salt concentration, are not acceptable to producers or consumers. A concentration of 15% NaCl in the brine has been considered as most suitable for wet-curing when taking into account the factors mentioned above (Nguyen *et al.*, 2010; Du *et al.*, 2010). We chose to use 15% salt concentration for curing in this study.

LF-NMR proton relaxatio A continuous distribution analysis of T_2 relaxation for meat samples without brining (0 h) and with brining for 5 h is shown in Fig. 1. It shows that T_2 relaxation was characterized by four components, each likely identified as follows: a minor component (T_{2b}) between 1 and 10 ms reflecting bound water, a larger component (T_{2f}) between 30 and 100 ms representing immobilized water, a third component (T_{22}) between 100 and 300 attributed to free water (Bertram *et al.*, 2003) and the last and largest component (T_{23}), between 1000 and 10000. The four components attributed to water tightly associated with

macromolecules, water located within highly organized protein structures, extra-myofibrillar water (Bertram *et al.*, 2003), and water in brine, respectively.

The changes in T_2 relaxation times during curing treatment of the three major components are shown in Table 1. Strong significant effects of curing on the T_2 relaxation times were found. With increasing times of curing up to 5 h, the components T_{2f} and T_{22} both increased whereas T_{23} decreased. It is generally considered that transverse relaxation times (T_2) reflect the bonding forces between water and meat tissue, especially the myofibrillar proteins, given their strong polar bonding. A high T_2 value suggests weak bonding force whereas a low T_2 value suggests the opposite. In the present study, T_{2b} remained constant at approx. 4 ms with the meaning that the bond water was stable. The increase in T_{2f} and T_{22} indicated that water in meat was more loosely bound with proteins and the myofibrillar components. On the contrary, the T_{23} corresponding to water in brine decreased indicating that water molecules in the brine increase physicochemical interaction with the proteins.

Fig. 2 shows the water distribution between brine and meat at various times of curing. The ratio of proportion of water in brine and meat in the curing system were 66% to 34% at 0 h, whereas it

Fig. 1. Representative distributions of T_2 relaxation times for meat cubes brined at time 0h (full line) and 5 h (dotted line) of curing in 15% NaCl solution. T_2 data were performed with a τ -value (time between 90° pulse and 180° pulse) of 400 μ s. The measurements were performed at 32°C , and data were acquired as 16 scan repetitions

Table 1. The changes of relaxation time during curing in 15% NaCl solution

Curing time(h)	$T_{2b}(\text{ms})$	$T_{2f}(\text{ms})$	$T_{22}(\text{ms})$	$T_{23}(\text{ms})$
0	$4.20 \pm 0.35\text{a}$	$49.77 \pm 0.00\text{a}$	$175.51 \pm 0.38\text{a}$	$2154.43 \pm 0.00\text{a}$
1	$4.16 \pm 0.13\text{a}$	$49.77 \pm 0.00\text{a}$	$205.43 \pm 8.34\text{b}$	$1833.14 \pm 40.69\text{b}$
2	$4.53 \pm 0.58\text{a}$	$52.25 \pm 0.00\text{b}$	$216.31 \pm 12.79\text{b}$	$1683.99 \pm 27.12\text{c}$
3	$4.28 \pm 0.31\text{a}$	$52.25 \pm 0.00\text{b}$	$216.47 \pm 3.34\text{b}$	$1629.75 \pm 0.00\text{c}$
4	$4.25 \pm 0.20\text{a}$	$55.57 \pm 0.83\text{c}$	$218.12 \pm 0.65\text{b}$	$1629.75 \pm 0.00\text{c}$
5	$4.60 \pm 0.68\text{a}$	$57.22 \pm 0.00\text{c}$	$232.16 \pm 2.18\text{c}$	$1629.75 \pm 0.00\text{c}$

Contents of nucleotide were expressed as mean standard deviation ($n = 3$). Mean with different superscripts in the same column indicate significant difference ($p < 0.05$).

changed to 63% to 37% after curing for 5 h. The changes in proportion of brine and meat indicated that water in brine decreased significantly while total water in meat increased.

Fig. 3 shows changes of the redistribution of water during curing process in the meat, the percentage of T_{2l} component were increasing significantly and the T_{2b} and T_{22} components were decreasing on the contrary. It was concluded that some amount of bound water and free water had been retained as immobilized water in meat.

Changes in water weight and Aw during brining The total weight changes (ΔM_t^o) and water weight changes (ΔM_t^w) were calculated by means of Eqs. (1) and (2):

$$\Delta M_t^o = [M_t^o - M_o^o] / M_o^o \times 100 (\%) \quad \cdots \cdots \text{Eq. 1}$$

$$\Delta M_t^w = [M_t^o \times X_t^w - M_o^o \times X_o^w] / M_o^o \times 100 (\%) \quad \cdots \cdots \text{Eq. 2}$$

M_t^o and M_o^o are the meat cube weight; X_t^w and X_o^w are the water weight fractions in meat, at sampling time t h and 0 h, respectively.

Fig. 4 and 5 show the increase in water weight and the decrease

in Aw during the 5 h curing process respectively. Large variations in both meat weights and Aw were observed over the brining process. The 5 h brining period resulted in a 9.5% increase in meat weight. And the increase in water weight was confirmed that meat gained water during curing. Aw was estimated to decrease from 0.96 to 0.91 during this time, with rapid decrease in the first 1 h. Aw is an important physical property of food which is relevant to its shelf-stability and represents the ‘water availability’ in a material (Lowe and Kershaw, 1995). The finding of decreasing of Aw is in agreement with that of Aliño (2010) who showed that Aw decreased even at different salt concentrations during curing.

The correlations between water weight change, Aw and the percentages of populations and relaxation times of the three water components are presented in Table 2. Water weight change has significant correlation with Aw, T_2 and P_2 indicating that as water increasing in meat, many changes occurred meanwhile. The highest correlation to water weight change was found for P_{2l} ($r = 0.99$) and P_{23} ($r = -0.99$), that means water was transferred from brine to meat during curing and the increasing water content in meat is

Fig. 2. Water distribution between meat and brine measured by NMR during curing of meat cubes in 15% NaCl solution. Each error bars indicate mean values $\pm$ standard error of three replicates. Values bearing different superscripts are significantly different ($p < 0.05$)

Fig. 4. The changes of water weight in meat cubes during curing in 15% NaCl solution. Each error bars indicate mean values $\pm$ standard error of three replicates. Values bearing different superscripts are significantly different ($p < 0.05$)

Fig. 3. The changes of three water components' proportion in meat cubes determined by NMR during curing in 15% NaCl solution

Fig. 5. The changes in Aw of meat cubes during curing in 15% NaCl solution. Each error bars indicate mean values $\pm$ standard error of three replicates. Values bearing different superscripts are significantly different ($p < 0.05$)

mostly due to the increase in the immobilized water component. The high negative correlation between P_{21} and P_{22} ($r = -0.99$) indicated that some free water were changing to the immobilized water. Aw is found correlated with T_2 and P_2 suggesting that the change of Aw may be caused by three water components exchange in meat. The strongest correlation to Aw was found for and P_{21} ($r = -0.91$), and this high negative correlation suggests that the decrease in Aw may be caused by the increase in immobilized water which has a lower Aw.

Changes in WHC and pH during brining The pressurization loss of samples shows a significant ($p < 0.05$) reduction (from 50.2 to 42.8) during the first 1 h (Fig. 6) and then slowly increased (from 42.8 to 49.1) during the remainder of the curing process. Finally, the pressurization loss becomes stable at a value about 49, which is similar to that of the raw meat. WHC increased initially and then decreased on the contrary. Correlations between pressurization loss and P_2 and T_2 of three water components are presented in Table 2. Pressurization loss showed no correlation with T_2 relaxation data.

Table 2. Correlation coefficients and levels of significance for the correlations between the water change, Aw, pressurization loss and the T2 time constants and corresponding proportion

	ΔM_t^w	Aw	pressurization loss	P_{21}	P_{22}	P_{23}	T_{21}	T_{22}	T_{23}
ΔM_t^w	1	-0.93**	0.084	0.99**	-0.97**	-0.99**	0.88*	0.87*	-0.92**
Aw		1	0.28	-0.91*	0.90*	0.95**	-0.70	-0.96**	0.99**
pressurization loss			1	0.14	-0.096	-0.027	0.437	-0.246	-0.272
P21				1	-0.99**	-0.99**	0.92**	0.87*	-0.90*
P22					1	0.98**	-0.92**	-0.84*	0.89*
P23						1	-0.88*	-0.92*	-0.95**
T21							1	0.69	-0.68
T22								1	-0.96**
T23									1

Level of significance: * = $p < 0.05$; ** = $p < 0.01$.

Fig. 6. The changes of Pressurization loss during curing in 15% NaCl solution. Each error bars indicate mean values $\pm$ standard error of three replicates. Values bearing different superscripts are significantly different ($p < 0.05$)

The change of pH is showed in Fig. 7. The pH of the raw pork meat in this article was about 5.56, after curing for 1 h, the pH decreased to 5.31, and then during the period of the following curing process, pH had slightly reduced to about 5.27. As NaCl transferred from brine to meat, the ionic strength increased and may destroy the chemical bond of protein structure, induced changes in protein conformation, and then covered up the basic group in protein.

The salting process is known to affect the WHC of meat. In the present study, the change of WHC may be affected by a combined effect of pH and NaCl. At 0 h, the pH of meat is 5.56 which is closed to the isoionic point (pH = 5.50) of meat and at this point meat cannot hold the water efficiently, so the pressurization loss is high; In the first 1 h curing course, pH decreased to 5.31 which is away from the isoionic point, at the same time with increasing content of NaCl in meat some proteins become solubilized causing the myofibrils to swell as a result of the increased influx of water (Whiting RC, 1988), both the two reasons led to an apparent

Fig. 7. The changes of pH during curing in 15% NaCl solution. Each error bars indicate mean values $\pm$ standard error of three replicates. Values bearing different superscripts are significantly different ($p < 0.05$)

Fig. 8. The changes of Pressurization loss during curing in 15% NaCl solution. Each error bars indicate mean values $\pm$ standard error of three replicates. Values bearing different superscripts are significantly different ($p < 0.05$)

increase in the WHC of meat, so at 1 h the pressurization loss is low; then during the following course, pH is nearly stable, so the absorbing of NaCl mainly affects the WHC. Zhou (2008) considered that the WHC is highest when concentration of NaCl in meat is 4.6% ~ 5.8%, after curing 2 h, the concentration of NaCl may increase more higher and break the microstructure of meat, so the WHC of meat reduced and the pressurization loss of meat increased.

Changes in thermal denaturation temperature of meat before and after curing The data in Fig. 8 shows the changes of thermal denaturation temperature of samples during the curing process. For raw meat heated from 20°C to 100°C, three peaks were observed with T_{max} values of 53.55°C, 63.9°C and 75.52°C, corresponding to myosin (I), sarcoplasmic proteins and collagen (II), and actin (III) (Graiver *et al.*, 2006). After curing for 5 h, the first and the third peaks disappeared whereas the second peak remained with a similar thermal denaturation temperature of about 65°C, but it was markedly reduced, indicated that the myofibril protein especially myosin and action may be dissolved and transferred from meat to brine with a phenomenon that the brine was becoming muddy after curing. It is more likely that the increase in T_{21} is due to an increase in the spacing between the myofibrils or shortening of the sarcomeres and the increase in T_{22} represent an increase in the space in extra-myofibrillar area (Pearce, 2011). This infers that the meat microstructure is swollen by the curing process.

It is known that there are complex changes occurring in meat when wet-cured in 15% brine (Nguyen *et al.*, 2010; Du *et al.*, 2010). The main change is the mass transfer between meat and brine of salt, water and together with small amounts of proteins and fat. When meat is exposed to brine, NaCl diffuse from brine to meat since the 15% salt concentration in brine is higher than that in meat. The penetration of NaCl into meat may induce proteins denaturation and solubilisation (Desmond *et al.*, 2006). The present study showed that after curing for 5 h, myofibrillar proteins including myosin (thermal denature at 55°C) and actin (thermal denature at 75°C) dissolved and then probably transferred to brine.

In addition, the denaturation of myofibrillar proteins may lead to higher swelling pressure in the meat cubes and then cause in changes in muscle microstructure so that the meat produces more spaces to retain water. There are two possible mechanisms accounting for myofibrillar swelling during curing (Offer and Trinick 1983): the first one is that binding of negatively charged ions increases the electrostatic repulsion between the myofilaments, and secondly, that removal of one or more transverse structural constraints in the myofibril allows the filament lattice to expand. Regarding water transfer during curing, water initially moves from the brine solution into the region of the meat close to the surface thus increasing the free water content. Then, intermolecular forces result in an increase of NaCl concentration, causing swelling of the meat microstructure. Water then continues to transfer into the meat myofibrils resulting in more intra-myofibrillar water during curing. On the other hand, the bound water also exchanges with immobilized water because of the denaturation of myofibrillar proteins. The net result is that the water gained by meat is mostly present as intra-myofibrillar water.

Conclusion

Using LF-NMR, the present study showed significant change in water dynamics in pork meat during immersing in a 15% NaCl solution. The curing process had a significant influence on water properties, state and distribution in meat. As water transferred from brine to meat, the increased water in the meat was mostly converted to immobilized water as a result of myofibrillar protein denaturation and the subsequent microstructural changes occurring as a function of the NaCl. The population of T_2 was related to the water weight changes as measured by the AOAC method. In addition, the changes in water state and in properties, indicated by NMR, would also affect the quality values such as Aw. WHC changed upon the combine effect of salt and pH. In conclusion, it is possible to obtain greater weight yield and improved quality during meat curing by controlling the process based on our theoretical knowledge of meat curing, as presented in this study.

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