The potential for predicting purge in packaged meat using low field NMR

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Abbreviations

CPMG, Carr-Purcel-Meiboom-Gill; LD, *longissimus dorsi*; *p.m.*, post mortem; PSE, Pale Soft Exudative; WHC, water holding capacity

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Abstract

The ability of NMR to predict purge from vacuum-packed pork that was stored for 9 days was investigated. T_2 relaxation was measured at 24 h post mortem (*p.m.*) and again after 9 days of chilled storage. NMR measurements from day 1 *p.m.* were limited in predicting day-9 purge (|r| = 0.37-0.52). The root mean square error of linear regression (RMSD) for measuring day-9 purge using the relaxation time of intra-myofibrillar water (T_{21}) measured on day 1 *p.m.* (r = -0.46) was 1.31% (range: 1.15-7.69% purge), corresponding to ±2.62% (2 × RMSD) prediction error of purge with 95% probability. This indicated that for purge production rate, the distribution and mobility of water in meat on day 1 *p.m.* may be of little relevance. Further tests were conducted to explain this poor predictability, by taking NMR measurements of water mobility and distribution made on the same meat sample (taken at 96 h *p.m.*) every day, during a 9-day storage period. By analyzing the T_{21} and T_{22} domains every day, it was revealed that during the first 5-day of storage, water (86%) moved from intra-myofibrillar space to extra-myofibrillar space. However, this movement did not result in detectable drip. A major liquid loss followed between days 6 and 7 and ceased day 8. This complexity of the water movement between domains during storage may explain the poor predictability of day-9 purge using NMR measurements from day 1.

Key words: Purge; Water holding capacity; NMR; Storage; Porcine *longissimus dorsi* muscles; Meat structure

1. Introduction

The drip loss of meat during chilled storage depends on the amount of water that is available and the ease with which the water can exit the muscle structural network (Warner, 2014). The drip loss of meat is influenced by four major structural factors: 1) the degree of myofibrils shrinkage during rigor and myofibrillar interfilamentous spacing; 2) the permeability of the cell membrane to water; 3) the degree of cytoskeletal protein degradation and 4) the development of drip channels and extracellular space (Hughes, Oiseth, Purslow, & Warner, 2014). Water holding capacity (WHC) is very often measured as drip loss; i.e. the weight loss percentage of a meat sample after a defined period of chilled storage (24 or 48 h) in specifically designed holder (Christensen, 2003) or in a plastic bag (Honikel, 1998), where the meat has no physical contact with drip. Purge, in this paper, refers to the weight loss from meat during storage, where the meat is in contact with the fluid. Purge is the accumulation of a red aqueous solution of proteins in packaged, refrigerated meat and relates to what would be visible to a consumer. Drip loss and purge are important variables relating to profitability and quality of meat products and are highly relevant to both meat industry and consumers. However, these two variables have been reported to be controlled by different processes. Drip loss shows the WHC of meat at certain time post mortem; whereas purge is likely to be the accumulative effect of changes in WHC during storage. Several experiments have recorded a change in drip loss from 24 h *p.m.* up to 14 days p. m. (Joo, Kauffman, van Laack, Lee, & Kim, 1999; Kristensen & Purslow, 2001; Moeseke & Smet, 1999; Straadt, Rasmussen, Andersen, & Bertram, 2007) using different methods (48 h Honikel bag method or 24 h centrifugation). In general, the measured drip loss (%) peaked at around 48 h post mortem and subsequently decreased. The daily drip loss post mortem seems to be animal/sample dependent. For instance, in the work of Kristensen and Purslow (2001), the average centrifugation loss of 6 muscles reached its maximum on day 7 *p.m.*, whereas the average centrifugation loss of 4 other muscles in the same work reached its maximum on day 3 *p.m.*

There exist two explanations regarding the decrease in rate of drip loss (increase in WHC) in meat that is stored in contact with its own drip:
1). The reduction in drip loss with sampling time post mortem is a result of "leaking out", i.e. the meat with poor WHC (i.e. pale soft exudative meat, PSE) will lose relatively more water early postmortem (Joo et al., 1999; Moeseke & Smet, 1999). This leaves limited water available for dripping in later stages. Meat with a normal WHC has relatively more water to lose in later stages and this water serves as a "drip reservoir" that will eventually produce similar amount of drip as meat with poorer WHC (Joo et al., 1999).

2). Degradation of cytoskeleton proteins can result in an increase of WHC later post mortem (Huff-Lonergan & Lonergan, 2005; Kristensen & Purslow, 2001; Melody et al., 2004; Straadt et al., 2007). Cytoskeleton proteins (represented by vinculin, desmin and talin) gradually degrade during 10-day *p.m.* storage period (Kristensen & Purslow, 2001). The inter-myofibrillar linkages and costameric connections are removed, and myofibril shrinkage becomes energetically less favorable. The flow of water into the extracellular space ceases, and previously expelled water can to some degree reverse, and support swelling of the myofibrils. The intramyofibrillar structure has been shown to be more homogeneous after 14 days of storage using a confocal laser scanning microscopy, which supported this hypothesis (Straadt et al., 2007).

There have been very few articles investigating the prediction of purge using data obtained early post mortem (Bidner et al., 2004; Calkins, Holthaus, Johnson, Eskridge, & Berg, 2005; Huff-Lonergan & Lonergan, 2005). As summarized by Huff-Lonergan & Lonergan (2005), one study have studied using the desmin degradation on day 1 *p.m.* to predict purge loss over 7 days using stepwise regression models. It was found that desmin degradation accounted for only 24.1% variation of purge. Similarly, another study also showed poor prediction of purge using several measurements (21 % variation explained), which aimed at predicting 21-day purge in vacuum packaged whole pork loins using models based on variables measured early *p.m.* (including season, fat depth, muscle depth, hot carcass weight, color, pH and electrical impedance) (Calkins et al., 2005). It seems, therefore, that purge is challenging to predict due to the complexity of purge production process. Zarate and Zaritzky (1985) studied the effect of storage conditions on purge production in the package along storage time (until 22-day storage) in packaged refrigerated beef (cut at 48h *p.m.*). Two temperatures (0 and 4 ˚C) and two films (low density polyethylene and EVA/SARAN/EVA coextruded film) were studied and compared. During the first 24-hour storage (induction period), the purge (%) increased nonlinearly, and then the increase followed a reduced but constant rate. Similar results have been reported by Moeseke and Smet (1999) that the dripping rate decreased after 48 h post mortem. In addition, purge percentage was found to be linearly correlated to the equivalent area/unit volume ratio of the sample (Zarate & Zaritzky, 1985). Their work also suggested that the water that turned into purge during storage was located extracellularly and extramyofibrillarly, and the purge was mainly produced by gravitational force since the purge (%) rate is constant after induction time (Zarate & Zaritzky, 1985). They also refuted that diffusion is to explain the purge production, since a decreasing rate should be expected (Zarate & Zaritzky, 1985).

Since WHC increases with storage time, the WHC difference between meat with high or low initial WHC might decrease significantly towards later storage period, as shown in the study using meat with four different quality groups (Joo et al., 1999). However, the results showed that the meat with initial lower WHC (i.e. PSE) still had lower WHC on day 6 *p.m.* than meat that had a higher initial WHC. It is then reasonable to suggest that the accumulated purge of meat having an initial low WHC might be relatively high. This change in drip loss rate with time might make purge prediction difficult and demand methods with high and relevant analytical precision.

NMR is a powerful tool to study water mobility and distribution, and has been used extensively in studying meat structure and WHC. However, to the best of our knowledge, no studies have addressed the possibility of using NMR to measure purge. In this paper, we explored the ability of low field NMR and other measurements/variables obtained at or before 24 h *p.m.* to predict purge from pork muscle after vacuum-packed storage for 9 days. The 9-day storage period was chosen because it is the average storage time used for fresh meat cuts before displayed in retail stores according to Norwegian meat industry. The correlation between purge and variables obtained on samples after 9-day storage was also studied in order to: 1) determine the predictability of purge on day 9 from NMR measurements on day 1; 2) understand the purge production mechanism during the same number of days.

To support 1) and 2) the measurement error of the NMR instrumentation also needed to be verified to determine if NMR can measure a difference in water content between 80 % and 75 % water.

2. Materials and methods

2.1. Animals and sampling

In order to obtain meat samples with reasonable WHC variation, 18 pigs were selected from 2 different slaughterhouses (Tønsberg and Oslo, Norway) based on their meat percentage/ back fat thickness during three weeks. The chilling rate affects drip loss and this can vary due to the meat percentage/ back fat thickness. The animals were, therefore, selected to give variation in fat thickness and two different chilling methods were carried out in the two slaughterhouses. The pigs used had carcass weights between 56.1 to 100.1 kg. Breeds used were LYDD (25 % Landrace, 25 % Yorkshire and 50 % Duroc) and LYLL (25 % Yorkshire and 75 % Landrace). The pigs were stunned in an atmosphere with 90% carbon dioxide and slaughtered. At Tønsberg slaughterhouse, the carcasses were cooled for 30 min in the shock-cooler/freezer and then chilled down to 7 ˚C for 18 hours. At Oslo slaughterhouse, the carcasses were cooled for 18-20 h to below 7 ˚C, in a cooling room at 0-1˚C. The left porcine *longissimus dorsi* (LD) muscles were removed. Connective tissue and fat were carefully trimmed around the muscle.

The LD muscle from each animal was divided into two sections based on location (denoted L1 and L2, Figure 1a) with some space discarded between L1 and L2 (shown in grey, Figure 1a). The samples were treated as separate samples since a difference of WHC (as drip) has been reported between cranial and caudal ends (Taylor & Dant, 1971). For each location (L1 or L2), the muscle was divided as shown in Figure 1b on day 1 *p.m.*

In the study of the effect of storage time (section 3.3), six boars from Landrace and Duroc breed were randomly selected. The LD loins were cut at 96 h *p.m.* One sample was taken from each animal, resulting in a total number of six meat samples.

2.2. Purge measurement

On day 1 p. m., a chop of 12 cm in thickness (for L1 and L2 each) towards cranial end was divided, weighed (M_0, of 348.21-860.55 g) and vacuum packed using a Intevac vacuum packing machine with internal programming level 6 (Bissendorf, Germany) in a plastic bag (shown as purge in Figure 1b). The vacuum packed muscles were stored at 4 ˚C until day-9 post mortem; surface dried with tissue paper and weighed again (M). Purge (%) was calculated as the weight loss in percentage of the initial muscle weight (Purge (%) = 100 x (M_0-M)/ M_0). Purge values varied between 1.15% and 7.69 % (Table 1).

2.3. pH and color measurements

The muscle pH was measured at different times post mortem (45 min, 5 h, 24 h and day-9). The pH at 45 min and 5 h *p.m.* was measured by placing a Knick Portamess 752 electrode (Berlin, Germany) approximately in the middle of the loin. The pH at 24 h and day-9 *p.m.* was measured on the sample using Beckman Φ31 pH Meter (Brea, USA). The sample used for purge measurement on day-9 post mortem was divided according to Figure 1c. Color parameter including L*, a* and b* were determined using a Konica Minolta Chroma meter CR-400 (Tokyo, Japan) after 1 hour blooming, with the meat samples exposed to air, unwrapped. Three measurements were taken for each slice. Relevant statistics for pH at different time post mortem and color values are shown in Table 1.

Table 1. Ranges, means and standard deviations of chemical-physical parameters of porcine *longissimus dorsi* samples.

	Range	Mean	Standard Deviation
pH 45 min (n=18)	6.09-6.73	6.46	0.16
pH 5 h (n=12)	5.61-6.09	5.90	0.15
pH D1 (day 1)	5.26-5.63	5.43	0.10
pH D9 (day 9)	5.30-5.47	5.39	0.04
Purge (%, day 9)	1.15-7.69	3.71	1.46
L* (day 9)	52.41-61.12	56.92	2.10
a* (day 9)	6.32-11.20	8.30	1.37
b* (day 9)	4.80-8.32	6.10	0.73

Note: the number of samples (n) was 36 unless otherwise stated

2.4. NMR measurement

Transverse relaxation (T_2) was measured on meat samples both day-1 (Figure 1b) and day-9 (Figure 1c) *p.m.* using a Maran Ultra NMR instrument (Resonance Instruments, Witney, UK), operating at a magnetic field strength of 0.54 T, corresponding to a proton resonance frequency of 23 MHz. The NMR signals were recorded by applying a traditional Carr-Purcel-Meiboom-Gill (CPMG) pulse sequence (Meiboom & Gill, 1958) with τ = 150 μs, 12 K echoes and 16 transients. Three cylindrical samples (16ø x 22 mm, ~2.80 g) were cored using a sharp cork borer for each location (L1 and L2), and samples were gently inserted in closed Teflon sample holders (2.2 cm in length), and placed within the homogeneous part of the rf-coil. The samples were thermostated at 25 ˚C for 10 min before CPMG measurements were performed.

The influence of storage time on six meat samples (section 3.3) were also studied using another Maran Ultra NMR instrument (Resonance Instruments, Witney, UK) of the same magnetic field strength, but different sample size (~ 8ϕ x 10 mm, ~0.5 g). Each meat sample was suspended in the NMR tube with the fiber direction parallel to the cylindrical axis. Enough space (17 mm) was reserved between the bottom of the NMR glass tube and the muscle. A layer of parafilm was placed on the top of the muscle to avoid water evaporation. The CPMG signal response was acquired for each sample and stored every day during a 9-day storage period (corresponding to 4-13 days p.m.), performed at T = 6 °C and equilibrated at this temperature for 10 minutes before initiating any experiment. Samples were stored at 4 °C when not subjected to measurements. The NMR measurement was performed with a τ = 50 μs, 32 K echoes and 32 transients. The parafilm was found to not contribute to the NMR signal. After 9 days of storage, one CPMG experiment was performed on the drip fluid by lifting the sample tube manually (only the drip fluid was within the transmitter/receiver coil).

2.5. Data analysis

Distributed exponential fitting analysis was performed on the obtained T_2 relaxation data. A continuous T_2 relaxation time distribution $dI/d\log(T_2)$ was first derived from the CPMG signal response using Maran Ultra algorithm (RI Win-DXP software release version 1.2.3, Resonance Instruments, Witney, UK), which was described by Bertram et al. (Bertram, Dønstrup, Karlsson, & Andersen, 2002). I is the signal intensity of the NMR relaxation curve. Then a relaxation rate distribution $F(R_2)$ was obtained using the following transformation:

$$F(R_2) = \frac{dI}{dR_2} = \frac{dI}{d(LogT_2)} \cdot \frac{d(LogT_2)}{dR_2} = -\frac{T_2}{\ln 10} \cdot \frac{dI}{d(LogT_2)} \text{ with } R_2 = 1/T_2 \quad (1)$$

Three peaks were observed for all samples reflect the bound-, immobilized- and free water, respectively. The overall relaxation distribution takes the form:

$$F(R_2) = \sum_{i=0}^{2} I_i F_i(R_2) \quad (2)$$

where I_i represents the signal intensity and $\overline{R}_{2i}$ represents the "mean" relaxation rate of component "i", i.e.:

$$\overline{R}_{2i} = \int_0^\infty R_2 F_i(R_2) dR_2 \Big/ \int_0^\infty F_i(R_2) dR_2 \quad (3)$$

where i = 0, 1 or 2, and $\overline{R}_{20} > \overline{R}_{21} > \overline{R}_{22}$. Using a distribution function written in Microsoft Excel 2010 (Microsoft Corporation, WA, USA), the derived relaxation rate distributions were closely fitted. Only the domains with the longer relaxation times (T_{21} and T_{22}) changed during storage (Hansen & Zhu, 2015), and were further discussed. The relaxation times T_{21} and T_{22} correspond to intra-myofibrillar water and extra-myofibrillar water, respectively. The integrated areas of relaxation populations were normalized by sample mass (A_{21} and A_{22}), corresponding to T_{21} and T_{22}.

Correlation coefficients between variables ($P < 0.05$) were calculated using OriginPro 2016 (OriginLab Corporation, MA, USA).

3. Results and Discussion

3.1. Univariate Correlation Analysis

The Pearson correlation coefficients (r) for the measured variables can be seen in Table 2. Purge (%) was found to be better correlated to the following parameters: pH D1 (-0.46), pH D9 (-0.33), a* (-0.38), b* (-0.42), T_{21}-D1 (-0.46), T_{22}-D1 (-0.37), A_{21}-D1 (-0.43), A_{22}-D1 (0.52) and T_{21}-D9 (-0.70). Correlations between ultimate pH (pH D1) and purge in vacuum packages (7-day) have been reported with a similar correlation (r = -0.49) to the current study (Bidner et al., 2004). For color measurements, significant correlations were found between L* and b*, as well as a* and b* at $P<0.05$ (Table 2). Significant positive correlations regarding same color parameters (L* and b*, a* and b*) have been reported for beef *longissimus thoracis* muscle by Leroy et al. (Leroy et al., 2003). Interestingly, among all the color parameters, only a* (measuring redness to greenness) correlated better with the NMR parameters. This may indirectly be due to pH variation (Table 1). Another interesting observation was the decrease in pH *p.m.* when an increase was expected due to protein degradation.

Table 2 Pearson correlation coefficients (r) between measured variables.

	pH 5 h	pH D1 (day 1)	pH D9 (day 9)	Purge (%)	L*	a*	b*	T_{21}-D1 (s)	T_{22}-D1 (s)	A_{21}-D1	A_{22}-D1	T_{21}-D9 (s)	T_{22}-D9 (s)	A_{21}-D9	A_{22}-D9
pH 45 min	**0.64**	-0.07	-0.30	-0.29	0.09	-0.11	0.20	-0.26	-0.04	0.13	-0.20	0.02	-0.25	-0.12	0.12
pH 5 h		0.29	0.27	-0.32	-0.22	-0.01	0.19	-0.15	0.05	0.19	**-0.47**	0.39	0.06	-0.06	-0.03
pH D1 (day 1)			**0.52**	**-0.46**	-0.02	**0.59**	0.26	**0.51**	0.32	**0.33**	**-0.52**	**0.63**	**0.40**	-0.13	0.13
pH D9 (day 9)				**-0.33**	-0.28	0.30	-0.06	**0.40**	**0.36**	0.31	**-0.43**	**0.54**	**0.54**	-0.07	0.10
Purge (%)					-0.22	**-0.38**	**-0.42**	**-0.46**	**-0.37**	**-0.43**	**0.52**	**-0.70**	-0.29	0.03	-0.28
L*						0.01	**0.41**	0.04	0.21	-0.04	-0.03	-0.01	-0.16	0.13	0.06
a*							**0.49**	**0.51**	**0.39**	**0.44**	**-0.46**	**0.54**	**0.46**	-0.02	0.16
b*								0.14	0.15	0.21	-0.26	0.24	0.11	0.03	0.12
T_{21}-D1 (s)									**0.60**	**0.52**	**-0.50**	**0.65**	**0.62**	0.12	-0.10
T_{22}-D1 (s)										**0.73**	**-0.72**	**0.52**	**0.58**	0.18	-0.10
A_{21}-D1											**-0.84**	**0.43**	**0.54**	0.03	-0.17
A_{22}-D1												**-0.59**	**-0.61**	-0.06	0.15
T_{21}-D9 (s)													**0.56**	0.24	0.13
T_{22}-D9 (s)														**0.41**	**-0.49**
A_{21}-D9															**-0.54**

Notes: T_{21}-D1 and T_{22}-D1 are relaxation time constants measured on day 1 *p.m.* A_{21}-D1 and A_{22}-D1 are areas of each domain normalized by sample mass, measured on day 1 *p.m.* T_{21}-D9 and T_{22}-D9 are relaxation time constants measured on day 9 *p.m.* A_{21}-D9 and A_{22}-D9 are areas of each domain normalized by sample mass, measured on day 9 *p.m.*

$P < 0.05$, all the significant correlation coefficients are marked in bold.

The longest spin-spin relaxation time (T_{22}) corresponds to water that resides outside the myofibrillar protein network, which is most susceptible to dripping (Bertram, Purslow, & Andersen, 2002). T_{22} has been investigated as a reference value for WHC (at 24 h *p.m.*) in a previous study, which was based on drip loss (Zhu et al., 2016), but T_{22} did not show a good prediction ability towards purge after storage. The correlation coefficient between T_{22} measured on day-1 *p.m.* and purge was -0.37 (Table 2) and therefore nominally lower than the correlation given for T_{21} above (r = -0.46, RMSD = 1.31%, of 1.15-7.69% purge). In principle this indicated that purge can be predicted as ±2.6% (2 x RMSD) with 95% probability. The normalized area of the two domains, A_{21}-D1 (r = -0.43, RMSD = 1.33%, of 1.15-7.69% purge) and A_{22}-D1 (r = 0.52, RMSD = 1.27%, of 1.15-7.69% purge) also correlated to purge, which indicates that both domains are relevant regarding purge production. The measurement error in purge using the current method is unfortunately unknown. However, error of purge loss on beefsteaks (~0.23 kg) was estimated to be 3-4 % (Elam, Brooks, Morgan, & Ray, 2002). The error in water mass (g) predicted by NMR total intensity measured on 20 meat samples from 1 loin was 0.019 g (~ 2.150 g H_2O in meat sample of mass 2.87 g, r = 0.9945), assuming 75 % of water in the meat samples (data not shown). This indicates that NMR has the ability to discriminate meat samples that has water content difference of 1.77%, with 95% probability. This actually suggests that the purge can be predicted but that the major reason for the lack in predictability of NMR variables is due to the low reproducibility of NMR on heterogeneous samples like meat. This could be improved using the average of several samples or increasing the size of the samples.

The shorter spin-spin relaxation time (T_{21}) corresponds to intra-myofibrillar water. T_{21} could not alone predict purge (Table 2) with high accuracy. Multivariate models, using different variables in Table 2, were also investigated, but no improvement in correlation was obtained. One explanation as to why it is difficult to predict purge from early post mortem measurements is that there is a sum of events related to water mobility that occur during the storage period (Moeseke & Smet, 1999), which results in changes in the drip rates with storage time (i.e. 1-9 days). To explore these further, T_2 characteristics from day 1 and day 9 were compared.

3.2. T_2 characteristics on day 1 and day 9 *p.m.*

As shown in Figure 2, both T_{21} and T_{22} decrease after 9-day storage (slope <1, $p<0.05$). The change in T_2 relaxation times reflects the change in mobility of water molecules, shorter T_2 indicated water that has lower mobility and *vice versa*. The decrease in T_{21} and T_{22} indicates a decrease in both intra-myofibrillar and extra-myofibrillar water mobility. Straadt et al. (2007) also observed a decrease in T_{21} after 7-day storage, as well as a change in width of the T_{21} distribution. The T_{21} width in their studies decreased at day 7 (and day 14) compared to day 1 *p.m.*, indicated a more homogeneous characteristics of intra-myofibrillar water, presumably due to swelling (Straadt et al., 2007). Similarly, a decrease in T_{21} width (calculated as full width at half maximum height) has been observed in the current study when comparing day 1 and day 9 post mortem (data not shown). T_{22} has been shown to reflect the width of gaps between meat fiber bundles, and to correlate positively with drip loss measured at short time intervals (Tornberg, Andersson, Göransson, & von Seth, 1993). Thus the observed decrease in T_{22} after 9-day storage indicates a decrease in drip loss or, in other words, an increase in WHC. The range of T_{22} among samples decreased after 9 days of storage, which indicated that the spread in WHC of meat samples has decreased. Our results are in accordance with the findings of Joo et al. who has also reported a reduced spread in WHC after storage (Joo et al., 1999). The area of T_{21} and T_{22} was normalized by sample mass, and the difference was calculated between day 1 and day 9. There was an average increase of T_{21} area by 2.4%, and

an average decrease of T_{22} area by 36.1% observed on day 9 compared to day 1 p. m. The relative small change in T_{21} area is somewhat expected, since the water representing the T_{21} domain (intra-myofibrillar water) is about 85% of total water in the meat, a big absolute change might appear to be small when it is shown on the relative scale. The decrease in T_{22} domain is most likely a result of fluid dripping out. Drip formation mechanism early post mortem has been discussed by Tornberg et al. (2000) and Bertram et al. (2004). NMR characteristics were measured on porcine *longissimus dorsi* muscle continuously for 24 hours. They suggested that during early post mortem, muscle cells swell within 2-3 h *p.m.* (increase in T_{21}), and then expel water into extra-myofibrillar space (increase in T_{22} area) which reflect potential drip loss. Unlike early p. m., structural changes during storage for a longer period is different. As explained by Kristensen and Purslow (2001), within 24 h storage, water flows from intra- to extracellular water compartment due to pressure. After several days of storage, the shrinkage of myofibrils halted (Kristensen & Purslow, 2001), due to the slow degradation of cytoskeletal connections, and extracellular water was then able to flow into myofibrils. The tendency for an increase in the area of T_{21} domain (intra-myofibrillar water) support inflow of water at longer storage times (9-day storage). During the 9-day storage, the meat was vacuum packed, and the meat surface was in contact with the drip fluid at all times. It is thus suggested that the uptake of extra-myofibrillar water became possible not only from T_{22} water domain, but also from drip fluid if in contact with the meat. To verify this hypothesis, an experiment was designed and results presented in section 3.3.

3.3. T_2 characteristics during storage

In order to study the effect of storage time on continuous purge production and verify that the area change of myofibrillar water was partly due to the inflow of water from the extracellular space, six LD meat samples taken from six different boars were inserted into six NMR tubes and measured every day during storage at 4 ˚C for 9 days. The six animals selected had ultimate pH in the range 5.54-5.56, and 24h EZ-DripLoss in the range 4.3-6.5%. The relaxation distribution of one of the six meat samples during storage is shown in Figure 3. Since enough space was reserved between meat sample and the bottom of the NMR tube, drip fluid could flow freely to the bottom of the NMR tube and did not interact with the meat after it had dripped. The sample ends were not fixed which enabled natural muscle shrinkage.

The mean T_2 values, their mean areas and the mean decrease in total area (%) of six samples are plotted along the storage period of 9 days in Figure 4. The 95% confidence intervals were also calculated and included. Figure 4 a and b show the decrease in average T_2 during 9 days of storage, which is in accordance with the observation mentioned in section 3.2, indicating more restricted mobility of water in both domains. The average decrease in T_{21} followed a constant rate until day 8 storage, after which a slight increase of T_{21} was observed. A noticeable decrease in averaged T_{22} took place during the first 5-day storage. The area of each domain was also plotted along storage time (Δ in Figure 4 a-b). The accumulated decrease in the area of T_{21} and T_{22} domains was considered to be drip and was plotted against storage time in Figure 4 c. A linear relationship was found between the storage time and drip production ($r = 0.80$, RMSD = 1.81% with a purge range of 0 - 9.53%), but the movement of water in the compartments is not linear (Figure 4 a-b). The change of area of the two domains indicating water movement along storage time can be divided into three phases (shown as 1-3 in Figure 4), and will be addressed accordingly.

The first phase was the exchange between intra- and extra-myofibrillar water, took place from day 1 to day 5. The area of the T_{21} domain decreased while the area of the T_{22} domain increased from day 1 to day 5 (Δ in Figure 4 a-b). The increase in the area of the T_{22} domain

accounted for 86% of decrease in area of T_{21} domain on the day 5 of storage. The area changed in both domains and indicated that water movement within the first 5 days of storage was mainly water exchange between domains. This is illustrated by a slow decrease in the total area loss (Figure 4 c), i.e. slow drip loss. This observation is not consistent with the findings of Zarate and Zaritzky (1985), who reported a high purge production rate during the first 24 h storage, followed by a lower and then constant rate after 5 days. The difference can be explained by the difference in sample history and sample preparation. The sample in this study was cut at 96 h *p.m.*, while in Zarate and Zaritzky (1985), the samples were cut at 48 h *p.m.* The initial fast purge loss may have been released in current experiment right after cutting. The experimental setup by Zarate and Zaritzky (1985) was meat wrapped in plastic film, which enabled the inflow of water from purge fluid, while in the setup in this study; the meat sample was separated from purge fluid. The second phase was the extra-myofibrillar water being releases as drip (day 5-7). In this phase, both T_{21} and T_{22} area decreased continuously (Figure 4 a-b). Significant purge occurred during this phase, indicated by the decrease in the total area (Figure 4 c). In the third phase, the water flowing from both domains into drip fluid ceased. Both T_{21} and T_{22} area, and the decrease in total area loss remained constant on day 8- and day 9- storage. Interestingly, there is a slight increase (~2%) in T_{21} time constant on day 9 compared to day 8. The T_{21} value indicates the average distance between a water molecule and the protein surface (Wahlgren & Tornberg, 1996), and increased T_{21} thus indicates somewhat longer average distance. This might be caused by liquid inflow from the extra-myofibrillar space into the intra-myofibrillar space due to degradation of cytoskeletal structure. Although the mean T_{21} area and mean T_{22} area showed no obvious changes, an obvious increase of T_{21} area was observed on day-8 storage for some individual samples. The inflow might be more pronounced if the meat sample is in contact with purge fluid, but this topic needs to be further investigated. The relaxation distribution of the drip fluid in the bottom of the NMR tube was also analyzed at the end of the experiment. There was mainly one domain present with a relaxation time of 0.216 s, which resembles T_{22} in meat.

4. Conclusions

A number of quality parameters measured early postmortem appeared to correlate with purge measured on day 9 *p.m.* T_{21} measured on day 1 *p.m.* correlated negatively to purge ($r = -0.46$, RMSD = 1.31% with a purge range of 1.15-7.69%). Area of both T_{21} ($r = -0.43$, RMSD = 1.33%, of 1.15-7.69% purge) and T_{22} domains ($r = 0.52$, RMSD = 1.27%, of 1.15-7.69% purge) correlated to purge, i.e. both domains contributed to purge. However, the prediction ability was limited, showing that water mobility and distribution on day 1 *p.m.* might be of little value with regards to purge production.

Further analysis on six meat samples (taken at 96 h *p.m.*) were measured daily using NMR to monitor the changes in water mobility and distribution in both T_{21} and T_{22} domains for 9 days. The results indicated complex water movement during storage, which might serve an explanation for the poor prediction of purge in the package from early p. m. data. The water movement can be divided into three phases. During the first phase (day 1-5), water movement was mainly due to a shrinking pressure, from intra-myofibrillar water space to the free water domain. Significant purging of this free water occurred during the second phase (day 5-7). In the last phase (day 7-9), the decrease in total area ceased, with both T_{21} and T_{22} area remained constant. However, a nominal increase was observed in T_{21} time constant on day 8, indicated possible structural changes.

In conclusion, it is believed that the complexity of water mobility and distribution during storage requires to be taken into account if robust predictions of 9-day purge are to be achieved. Initial investigation reveals that robustness may be increased by being more selective about when measurements are taken during storage, especially if the meat is in contact with its own drip water.

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