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Evaluating nuclear magnetic resonance (NMR) as a robust reference method for online spectroscopic measurement of water holding capacity (WHC)

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ABSTRACT

The potential of using NMR as a reference method for WHC measurement in porcine *longissimus dorsi* was investigated. The accuracy of NMR when measuring small water changes was assessed in a model system and in muscles. Visible/near infrared (Vis/NIR) and X-ray were used as potential online spectroscopic methods to assess WHC on 40 muscles. Drip loss and spin–spin relaxation were also measured. Calibration models were built using partial least squares regression (PLSR) with Vis/NIR or X-ray spectra as input and NMR or drip loss values as output. The slowest spin–spin relaxation time (T_{22}) showed higher correlation with both Vis/NIR ($R^2_{CV} = 0.66$) and X-ray spectra ($R^2_{CV} = 0.76$) than EZ-DripLoss values, demonstrating NMR has potential as a reference method for WHC measurement. NMR was more robust against variation along the length of the muscle when compared to the EZ-DripLoss method.

1. Introduction

Water holding capacity is one of the most important traits for meat quality both in fresh meat and in processed products due to economic and sensory reasons. Online measurement of the WHC is still a dream for the meat industry despite the amount of research conducted in this area. WHC is affected by genetics, physiological factors, rearing conditions and factors with regards to slaughter and further processing (Den Hertog-Meischke et al., 1997).

The available methods for WHC determination (Trout, 1988) are mostly destructive and time consuming. Alternatives to conventional methods are fast spectroscopic methods that have the potential to be implemented online. Spectroscopic techniques have been investigated for assessing WHC in meat but there are unresolved issues with repeatability and accuracy (Brøndum et al., 2000; Elmasry et al., 2011; Prevolnik et al., 2009). Also, an unavoidable aspect of spectroscopic methods is their need to be calibrated against other methods. Assuming good experimental design when developing calibration models, the accuracy of spectroscopic techniques for measuring any quality parameters depend on three main factors: 1) the natural heterogeneity of all biological materials; 2) the distinctiveness and variation of the features in the measured spectra; and 3) the accuracy and repeatability of the reference method. The first and second are unavoidable due to sample characteristics and composition. Previous works have shown that when different reference methods of WHC were used for Vis/NIR spectroscopy, a significant variation in the coefficient of determination of calibration (R^2cal = 0.004–0.71) and prediction error of lost water (0.36–3.5%) appeared (Brøndum et al., 2000; Forrest et al., 2000;

Abbreviations: CPMG, Carr-Purcel-Meiboom-Gill; DFD, dark, firm and dry; NMR, nuclear magnetic resonance; PLSR, partial least squares regression; PSE, pale, softs and exudative; SG, Savizky-Golay; SNV, standard normal variate; Vis/NIR spectroscopy, Visible/near infrared spectroscopy; WHC, water holding capacity.

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Hoving-Bolink et al., 2005; Leroy et al., 2003; Pedersen et al., 2003; Prevolnik et al., 2010; Prieto et al., 2008; Savenije et al., 2006), probably related to variation in accuracy and repeatability of reference methods as well as differences in sample size.

Energy dispersive X-ray (or X-ray spectroscopy) and Vis/NIR spectroscopy were investigated as potential online spectroscopic methods. X-ray spectroscopy provides information of the intermolecular forces in water and meat microstructure (Diesbourg et al., 1988; Kosanetzky et al., 1987), i.e. the distance between myosin and actin fibers in the muscle, which is related to WHC (Hughes et al., 2014; Offer and Trinick, 1983). The volume of myofibrils can change up to threefold due to the changes in the interfilament spacing, which generates the driving force for drip losses and WHC variation (Offer and Trinick, 1983). Encouraging results have been obtained using lab-based X-ray diffraction measurements (due to scattering) showing post mortem changes in the pork myofilament lattice (Diesbourg et al., 1988; O'Farrell et al., 2014). Vis/NIR spectral changes occur due to pH reduction and subsequent denaturing of proteins affecting light scattering.

The EZ-DripLoss method has been preferred in many labs because it is simple, inexpensive, sensitive (Rasmussen and Andersson, 1996) and has produced relatively high heritability values in the Norwegian pig breeding program (Norsvin, Hamar, Norway, 2006-present). However, even as a reference method, the EZ-DripLoss method is slow ($t \geq 24$ h), labor intensive and highly dependent on the operator. The prediction of WHC using EZ-DripLoss as reference method does not provide any information about the dynamics behind the water loss, and from what sort of structural changes occurred. Hence, there is a need for a faster, accurate and robust reference method for WHC measurements. Although pork *longissimus dorsi* muscle, often used for EZ-DripLoss measurements, is visually homogeneous, inherent heterogeneities exist throughout the muscle, and they increase towards the cranial end (Christensen, 2003). The sampling procedure for EZ-DripLoss, as developed by the Danish Meat Research Institute, involves WHC measurements on two samples within the same slice to define a WHC value (Danish Meat Research Institute, 2010). The EZ-DripLoss value is known to depend on the position along the longissimus dorsi muscle. Christensen (2003) reported drip losses at three positions (A, B and C in Fig. 2, Christensen, 2003) using 11–15 slices of LD muscles from 34 animals. A, B and C indicate dorsal, superficial and ventral positions on LD muscle, and position A and C are the two normal sampling positions in routine EZ-DripLoss measurement. At position A the drip loss decreased linearly about 50% with slice number, while no change in drip loss with slice number was observed at position C. The heterogeneity of small meat samples may even increase when sample handling cannot be fully controlled since water distribution is sensitive to pressure. Standardization of manual or mechanical sample handling is crucial to minimize errors for most methods including NMR.

NMR proton relaxometry has been used for quantitative measurement of different components in meat (total fat and moisture content) (Sørland et al., 2004). It provides information on the physical (distribution, compartmentalization) and chemical (mobility, interactions with macromolecules) properties of the water (Bertram and Ersen, 2004). This means that NMR relaxometry could be used to quantify the mobility and distribution of water in different meat domains (Bertram et al., 2001; Tornberg et al., 2000) and has the potential to quantify WHC rapidly. To be more specific, WHC can be measured by relaxation time of water associated with pores in muscle of different size (Trout, 1988). Renou and Monin (1985) were among the first ones to show correlations between NMR relaxtometry (T_1 and the population of T_{21}) and WHC assessed by pH paper imbibition technique. Later on, an extensive number of studies have reported that meat of different WHC from Pale, Softs and Exudative (PSE) to Dark, Firm and Dry (DFD) can be distinguished by NMR transverse relaxtometry as reviewed by Pearce et al. (2011, Bertram et al., 2002b; Tornberg et al., 1993, Tornberg et al., 2000). Unlike bulk water, the Carr-Purcel-Meiboom-Gill (CPMG) relaxation curve of meat appears to be multi-exponential, i.e., characterized by a distribution function of spin–spin relaxation times, T_2s, resulting from microscopic meat heterogeneity (Renou et al., 1989). The slowest component (T_{22}, 100–250 ms, ~10% of signal intensity) corresponds to mobile water outside myofibrils (Bertram and Ersen, 2004; Tornberg et al., 1993). Tornberg et al. (1993) suggested that T_{22} corresponds to extracellular water, i.e. water that is most susceptible to dripping. Although T_{22} has been reported to relate to WHC as determined by Honikel bag method with correlation coefficients of 0.60–0.75 (prediction error was not reported) (Bertram et al., 2002a), T_{22} has not been investigated as a reference value for WHC.

The objective of this paper is to determine the suitability of NMR as a reference method for a faster, online spectroscopic method to evaluate WHC based on three studies. Since there is interest in the meat industry to know the total amount of moisture, immobilized and free water in the meat products (Q-PorkChains, 2007–2011), the ability of NMR to determine the parameters is investigated. The accuracy of NMR to measure small changes in water in meat was assessed. The measurement error of two spectroscopic methods, Vis/NIR spectroscopy and energy dispersive X-ray transmission in combination with two possible reference techniques, the EZ-DripLoss method and NMR are investigated. In addition, the possibility of using NMR as a reference method for WHC determination is discussed. The accuracy and repeatability of the NMR is evaluated.

2. Materials and methods

2.1. Animals and sampling

Without specification, the pigs used in studies 1 and 3 were young boars from Landrace and Duroc breed, tested at the Norsvin boar test station (Ilseng, Norway) as part of an on-going breeding program. The boars not selected for semen production, were slaughtered and had carcass weights of around 95 kg. The animals were stunned in an atmosphere with 90% carbon dioxide. The carcasses were left at 15 °C for 5 min and then chilled to 1–3 °C for 96 h before transporting to a partial dissection line at Animalia (Oslo, Norway), where the porcine *longissimus dorsi* muscle was removed.

In the second study, comparing EZ-DripLoss method, NMR and other spectroscopic techniques, 400 pigs of Landrace and Noroc (50% Duroc, 25% Landrace and 25% Yorkshire) were slaughtered (at Tønsberg, Norway) during 4 days. In order to obtain a wider range of WHC, forty pigs were selected based on their breed and pH measured 6 h postmortem (pH = 5.47–6.75). Left porcine *longissimus dorsi* loins were obtained 24 h postmortem and cut as shown in Fig. 1a) for the different measurement techniques.

2.2. NMR relaxation measurements

In the first study, transverse relaxation (T_2) was measured on a series of H_2O/D_2O mixtures (H_2O, vol% = 0, 2.5, 8, 12.5, 37.5, 50, 62.5, 75, 87.5, 100) using a Maran Ultra NMR instruments (Resonance Instruments, Witney, UK), operating at a magnetic field strength of 0.54 T, corresponding to a proton resonance frequency of 23 MHz. Sample volumes were 0.54 mL (height < 10 mm). Deionized water was purified in an ELGA-purelab system (Veolia Water, Paris, France). Deuterium water (99.9 atom% D) was purchased from Sigma–Aldrich (St. Louis, MO, USA). The NMR signal