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Prediction of water holding capacity and pH in porcine *longissimus lumborum* using Raman spectroscopy

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ABSTRACT

The main purpose of this study was to investigate if Raman spectra recorded at the exact same position as drip loss measurements could improve prediction of drip loss in pork. One ventral and one dorsal cylindrical plug, cut from a standardized slice from *Longissimus lumborum*, were used to determine drip loss by EZ-DripLoss method and to collect Raman spectra, while ultimate pH was measured at another location. Partial least squares regression models were developed using spectra from each plug individually or averaged spectra from both plugs. The best models used spectra from the ventral plug, resulting in r^2_{cv}=0.75, root mean square error of cross-validation (RMSECV) = 1.27% and ratio of prediction to deviation (RPD) =2.0 for EZ-DripLoss and r^2_{cv}=0.72, RMSECV = 0.05 and RPD = 2.0 for ultimate pH. Results indicate that Raman spectroscopy can be used for rough screening of drip loss and pH in pork, and that the location chosen for collection of spectra can be very important for successful predictions.

1. Introduction

Water holding capacity (WHC) and postmortem decline of pH are two important quality indicators for pork. Ultimate pH (pH_u) signifies the point where pH stabilizes postmortem, and the magnitude of pH_u has been shown to contribute to the overall quality of pork. Correlations between pH_u and a range of different quality attributes have been documented, where some of the noteworthy ones include drip loss, tenderness, color and flavor (Huff-Lonergan et al., 2002). Measurements of pH early postmortem have also been correlated with drip loss, where higher pH was linked to lower drip loss (Warriss & Brown, 1987). WHC is a complex quality attribute for pork, where factors such as pH, extent of proteolysis (Huff-Lonergan & Lonergan, 2005) and chemical composition (Lawrie, 1985), contribute to the amount of exudate formed postmortem. Excessive drip loss is of course contributing to loss of revenue for meat processors, but WHC is also affecting other quality factors, such as eating quality (Hughes, Oiseth, Purslow, & Warner, 2014) and processing properties of pork (Torley, D'Arcy, & Trout, 2000). Measurements of pH and WHC are invasive and are often conducted as spot checks in industrial settings, thus information on an individual basis is not available to meat processors. Measurement of pH is usually conducted by inserting a glass pH-probe directly into the meat, while WHC often is measured by gravimetrically determining drip formed from a defined slice of meat by the bag method (Honikel, 1998) or from cylindrical subsamples within the muscle by the EZ-DripLoss method (Rasmussen & Andersson, 1996).

Development of rapid and non-invasive meat quality assessment methods for on-line or at-line application is consequently of interest to the meat industry, for amongst others meat classification, optimization of production procedures and as a tool in breeding programs. One technique which has gained momentum the last decade for implementation in processing plants is Raman spectroscopy. The technique is non-invasive, relatively rapid and it can be implemented in processing plants with little to no modification to already existing instrumentation. In addition, Raman spectroscopy can provide detailed information about proteins, lipids as well as minor constituents in meat (Li-Chan, 1996; Ostovar Pour et al., 2019). The earliest study using Raman spectroscopy for assessment of WHC in pork was in 2003, presenting very encouraging results (coefficient of determination from cross-validation (r^2_{cv}) = 0.98 and root mean square error of cross-validation (RMSECV) = 0.27%). However, the data set was very limited, including only 14 samples (Pedersen, Morel, Andersen, & Balling Engelsen, 2003). Research in

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Raman spectroscopy and WHC in pork was resumed in the 2010s with the development of a handheld and portable Raman spectrometer for at-line analysis (Schmidt, Sowoidnich, & Kronfeldt, 2010). Using this handheld instrument, models with r^2_{cv} ranging from 0.52 to 0.73 and RMSECV from 0.6 to 1.0% for drip loss and r^2_{cv} from 0.31 to 0.68 and RMSECV from 0.05 to 0.09 pH units for pH were developed (Scheier, Bauer, & Schmidt, 2014; Scheier, Scheeder, & Schmidt, 2015). Andersen et al. (Andersen, Wold, Gjerlaug-Enger, & Veiseth-Kent, 2018) used a large volume Raman probe to analyze several meat quality traits, including EZ-DripLoss and pH_u, where the PLSR models yielded an $r^2_{cv} = 0.49$ and RMSECV = 1.24% for EZ-DripLoss and an $r^2_{cv} = 0.52$ and RMSECV = 0.06 for pH_u. Results obtained with the handheld probe and large volume probe were similar, but both approaches were subject to a discrepancy between the area of spectroscopic analysis and reference measurement, respectively.

A recent review on utilization of portable and handheld Raman instruments in meat science concluded that there is a major lack of knowledge on how these systems really will work in practice, since most published studies do not employ proper test set validation schemes. The study also puts emphasis on the importance of representative sampling and the lack of reproducible reference methods in the field (Beganovic, Hawthorne, Bach, & Huck, 2019). Thus, the main aim of this work was to investigate the potential for Raman spectroscopy to predict drip loss and measure pH_u of fresh pork by recording spectra from the exact place where the drip loss measurements are made, i.e., the two plugs used for assessment of WHC by the EZ-DripLoss method, intending to improve sampling procedures. By analyzing the two plugs separately it was also possible to infer if there were differences in their individual suitability for prediction of meat quality.

2. Material and methods

Detailed information about materials and methods can be obtained in Andersen et al. (2018) and Gjerlaug-Enger, Aass, Odegard, and Vangen (2010). A summary and additional information are given in the following paragraphs. Of note, the only spectroscopic technique used in the current study was Raman spectroscopy.

2.1. Animals and meat quality analyses

A total of 101 boars (45 Norwegian Landrace and 56 Norwegian Duroc) were included in the study. Boars were slaughtered in four batches (batch 1 $n = 30$, batch 2 $n = 18$, batch 3 $n = 37$ and batch 4 $n = 16$). The animals were stunned with 90% CO_2, followed by exsanguination, scalding, splitting, cooling (−22 °C) and chilling (1 °C to 3 °C) until a core temperature of 7 °C was reached. All animals were cared for in line with laws, internationally recognized guidelines and regulations for keeping pigs in Norway (The Animal Protection Act of December 20th, 1974, the Animal Welfare Act of June 19th, 2009 and the Regulations for keeping of pigs in Norway of February 18th, 2003). The animals used in this study were boars from a test station kept as a routine by Norsvin breeding program guidelines. Meat quality data used in this study was part of routine collections, and samples were taken after regular slaughter, meaning no ethics committee approval was needed. No animal experiments have been conducted in the scope of this research.

Carcasses were transported to Animalia, the Norwegian Meat and Poultry Research Centre, and at 4- or 5-days postmortem, the loin muscle (LL – *Longissimus lumborum*) was dissected from the right side of the carcasses and used for measurement of water holding capacity, pH and to collect Raman spectra.

Assessments of drip loss were performed using the EZ-DripLoss method (Rasmussen & Andersson, 1996), henceforth referred to as DL. In short, two circular samples at fixed locations, one ventral (V) and one dorsal (D) sample (Fig. 1), were placed in drip loss containers (C. Christensen ApS, Denmark) and stored at 4 °C for 24 h. After storage, drip loss was calculated based on accumulated drip in the container.

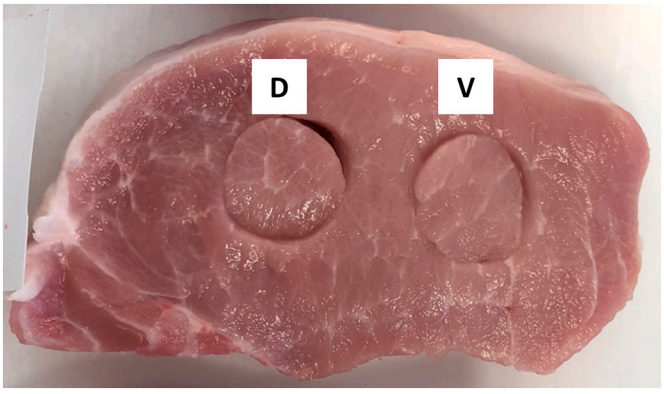

Fig. 1. Slice for determination of drip loss, location of dorsal (D) and ventral (V) plugs are indicated. Raman spectra were recorded on the surface facing up on the cylindrical plugs.

DL was measured for two adjacent slices from each muscle, where one slice was used for Raman spectroscopy and the other was used as a control for estimation of standard error of the DL measurement. Standard error of the reference measurement (SER) for DL was calculated as $SER = \left(\frac{\sum_{j=1}^{M} (Y_1 - Y_2)^2}{M} \right)^{\frac{1}{2}}$, where Y_1 and Y_2 represent drip loss from the two corresponding plugs (V vs. V and D vs. D) taken from adjacent slices, and M represents the total number of different samples used for the analysis (Mark & Workman, 2003). Number of samples for calculation of SER for DL was 142. SER for pH was calculated based on duplicate measurements from ten different samples, conducted as a part of the quality control at Animalia.

2.2. Spectroscopic analysis

Within one hour after being placed in the drip loss container, samples were removed carefully from the container, placed on an aluminum plate and Raman spectra were collected from a spot on the surface using a Kaiser RamanRXN2™ Multi-channel Raman analyzer (Kaiser Optical Systems Inc., Ann Arbor, MI, USA). The Raman system was equipped with a PhAT Probe which has a laser spot size of 6 mm. Spectra were recorded with laser power set to 400 mW in the range of 300–1890 cm^{-1} with 1.0 cm^{-1} intervals and exposure of 3 times 15 s. Power level at sample surface was measured to be approx. 210 mW with LaserCheck (Coherent Inc., Santa Clara, CA, USA). The sample was placed in the drip loss container after the spectrum was recorded.

2.3. Pre-processing of spectra

Each Raman spectrum was base-line corrected and fluorescence background was removed using a modified iterative polynomial curve fitting procedure (Modpoly) as described by Lieber and Mahadevan-Jansen (2003). The basis for the Modpoly method is a least-squares based polynomial curve-fitting function, where after the first iteration all Raman peaks with higher intensity than the polynomial curve are removed, and a new polynomial curve is calculated based on the remaining spectrum. This process is repeated until, ideally, there are no points that needs reassignment, and the resulting baseline spectrum is subtracted from the raw Raman spectrum. After baseline correction, the Raman spectra were subjected to standard normal variate transformation (SNV) (Barnes, Dhanoa, & Lister, 1989) to normalize the spectra.

2.4. Data analysis

Spectral comparison between V and D samples was done by comparing intensities at Raman shifts known to be mainly composed of amino acid contributions (e.g., tyrosine at approx. 830 cm^{-1} and phenylalanine at approx. 1003 cm^{-1}) or fatty acids (e.g., carbonyl stretch at approx. 1740 cm^{-1}). Intensities at these peaks were compared using a two sample *t*-test to determine if there were significant differences in intensities at the two different locations.

Partial least squares regression (PLSR) was used for determining linear relationships between DL and pH measurements and Raman spectra. PLSR models were cross validated by dividing the dataset in ten segments, leaving ten samples out at a time for error calculations. The segments were made to include each tenth sample, starting at sample 1, 2…10 for each segment, thus samples from each individual slaughter batch were included in every segment. Segments used in cross-validation were identical for all PLSR models. An uncertainty test was performed for the PLSR models to provide information about significant variables in the models and to aid in interpretation of models (Martens & Martens, 2000). The uncertainty test was also used to investigate if better models could be made by using only the significant variables. SNV corrected spectra were used for all PLSR models. The Raman spectrum from each plug (D and V) was used when making the model for all samples for DL, while the average spectrum from the two plugs were used to model pH using all samples.

The maximum value of r^2 for a calibration can be calculated from the following formula:

$r^2 = \frac{SD^2 - SER^2}{SD^2}$, where SD is the standard deviation for the reference measurement and SER is the standard error of the reference measurement. This is a useful metric to compare the final PLSR models r^2 to, because it indicates if there are possibilities for improvement of the model (Dardenne, 2010).

Ratio of prediction to deviation (RPD) values were calculated as outlined by Williams and Sobering (1993) and Williams (2014), using values for functionality factors as guidelines.

Baseline correction of Raman spectra were carried out using MATLAB (The MathWorks, Natick, MA), while SNV transformation was done in The Unscrambler® X version 10.4 (CAMO Process AS, Norway). PLSR analysis were conducted using The Unscrambler® X version 10.4 (CAMO Analytics AS, Norway). Statistical testing was done in Minitab® Statistical Software (Minitab LLC, Pennsylvania, USA).

3. Results and discussion

Results from reference analysis are summarized in Table 1, showing that the range of drip loss measured in this study was large, and the data was relatively well distributed (standard deviation/range = 0.20). The relative spread for pH_u was lower (standard deviation/range = 0.18), but the range was still large enough for modelling purposes and to be relevant for post-mortem evaluation of meat quality. The correlation coefficient between pH_u and DL was −0.68, showing a substantial relationship between pH_u and DL in the present study. Correlation coefficient between V and D samples for DL was 0.90, indicating that the two plugs from the same slice had comparable water holding capacity. The ratios between standard deviations of the reference measurements and estimated SER were relatively small, being 2.3 and 2.5 for DL and pH_u, respectively. When this ratio is small, the potential for developing good models decreases, as evidenced by the calculated maximum r^2 in Table 1. It is possible that the SER for DL was overestimated because it is practically impossible to measure the same sample twice, thus samples close to each other must be compared, giving a location error in addition to the pure measurement error.

Example pre-processed Raman spectra and difference spectra from the three samples with lowest and highest DL are shown in Fig. 2.A and 2.B, respectively. The shape of the pre-processed Raman spectra indicated that there were relatively minor differences in the spectra, but the difference spectrum revealed some regions with discernible deviations between low and high DL, which are discussed later. Results from PLSR models for DL and pH_u are summarized in Table 2. PLSR models for DL achieved r^2_{CV} and RMSECV in the ranges 0.56–0.75 and 1.27–1.68, respectively. The best model for DL was made using spectra and reference measurements from only location V, using only the significant variables from the uncertainty test, resulting in an RPD of 2.0, which is good enough for rough screening. Predicted vs. reference values for the best model for DL are shown in Fig. 3.A, indicating an even distribution of error in estimated drip loss for predictions throughout the entire range. PLSR models for pH_u achieved r^2_{CV} and RMSECV in the ranges 0.51–0.72 and 0.05–0.07, respectively. For pH_u, the best model was also made using spectra from location V only including significant variables from the uncertainty test, resulting in an RPD of 2.0. Predicted vs. reference values for the best model for pH_u are shown in Fig. 3 B. All models were improved by variable selection, but these models might not be the most robust models for prediction of new samples, because there is a risk of over-fitting when making models within a reduced variable space for a given sample set. The errors from models developed in this study were similar to what have been published earlier in studies on intact pork (Andersen et al., 2018; Scheier et al., 2014a; Scheier et al., 2015), but the relative ability to predict drip loss and pH_u increased, i.e., larger RPD values, meaning that the overall performance of Raman spectroscopy was slightly better in the present study.

Somewhat stricter validation procedures of PLS models were used to check the robustness of the models (results in supplementary table S1). Leave one slaughter batch out at a time cross-validation and test set validation using two slaughter batches for calibration and the other two for test set were investigated. Both methods gave larger RMSECV or root mean square error of prediction (RMSEP) than the previously described segmented cross-validation method. The increase in error was approx. 0.1 to 0.4 for DL and 0.01 to 0.02 for pH. This increase in error was caused by extrapolation and/or inflation of errors by a few outliers. E.g., by removing two suspected outliers from the DL validation set, RMSEP was reduced by approx. 25%. To address this more thoroughly, more samples and independent slaughter batches would be needed to ensure that most of the variation in spectra and reference measurements are included in validation segments. This was not the case when we used specific slaughter batches in calibration and test sets, since the data sets then spanned different variation.

Spectra from location V resulted in the best PLSR models for DL and pH_u, indicating that the ventral part of the muscle was best suited for spectroscopic analysis. The average Raman intensity for ventral samples was higher than for dorsal samples for almost the entire spectral range. In addition, ventral samples had significantly higher intensities ($p < 0.01$) for amino acid signatures (e.g., tyrosine and phenylalanine),

Table 1
Overview of results from reference analyses. Only one standard error of reference measurement (SER) and max r^2 were calculated for drip loss (DL). Values for DL are given as %, while values for pH are given as pH-units.

	N	*Mean*	*Min*	*Max*	*Range*	*Standard deviation*	*Estimated SER*	*Max r^2*
DL (All)	202	6.21	0.39	13.26	12.87	2.52	1.1	0.81
DL (V)	101	6.21	0.39	13.26	12.87	2.54		
DL (D)	101	6.22	0.47	12.54	12.07	2.52		
pH	101	5.51	5.22	5.77	0.55	0.10	0.04	0.84

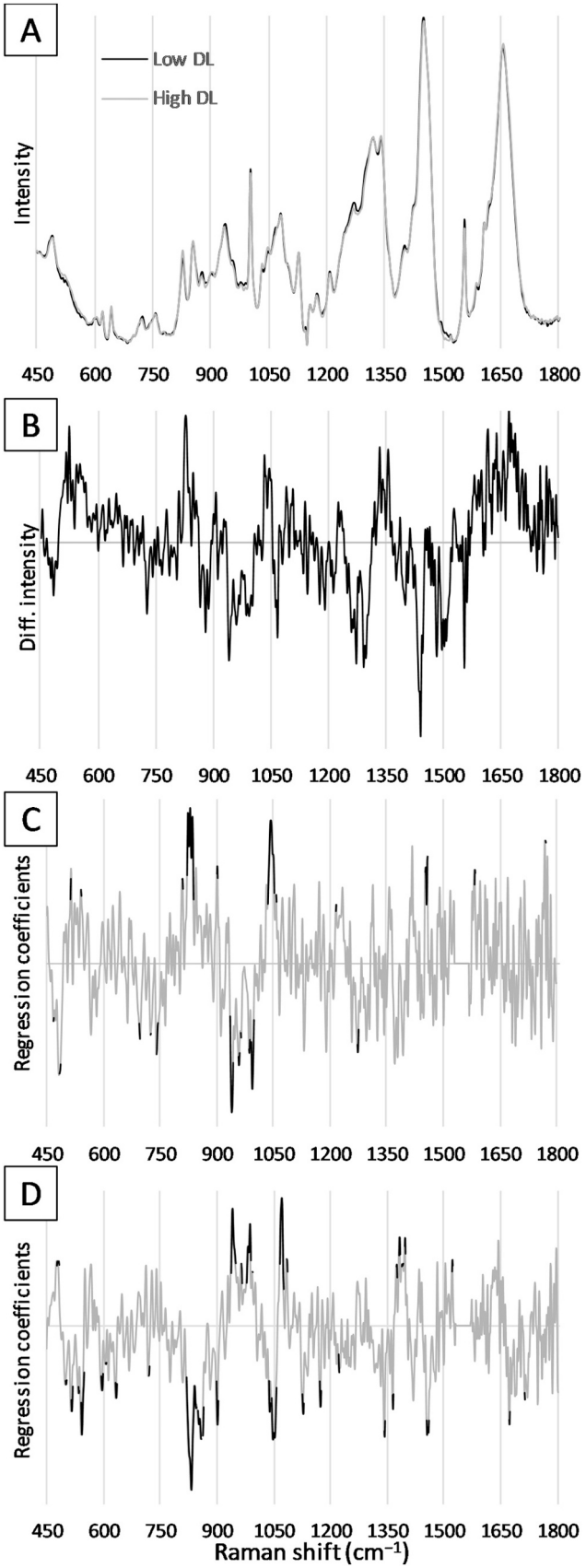

Fig. 2. A) Average pre-processed Raman spectra from the three samples with lowest and highest drip loss. B) Difference spectrum where the average Raman spectrum for low drip loss was subtracted from the Raman spectrum for high drip loss (same samples as in fig. A). C and D) Regression coefficients using the full Raman spectrum for PLSR models for EZ-DripLoss (C) and pH (D), where significant variables from uncertainty tests are marked in black. All Raman spectra and regression coefficients were taken from the ventral sub-sample.

Table 2
Performance of cross-validated PLSR models from Raman spectroscopy vs. reference measurement. Models from full Raman spectrum and models using only variables found to be significant by uncertainty test are included. Average Raman spectra from V and D samples were used for the pH (All) model.

		Full Raman spectrum				*Variables from uncertainty test*			
	N	r^2_{cv}	*RMSECV*	*Factors*	*RPD*	r^2_{cv}	*RMSECV*	*Factors*	*RPD*
DL (All)	202	0.61	1.57	5	1.6	0.71	1.37	4	1.8
DL (V)	101	0.57	1.67	7	1.5	0.75	1.27	6	2.0
DL (D)	101	0.56	1.68	5	1.5	0.69	1.41	4	1.8
pH (All)	101	0.57	0.07	5	1.4	0.70	0.05	5	2.0
pH (V)	101	0.59	0.06	5	1.7	0.72	0.05	4	2.0
pH (D)	101	0.51	0.07	5	1.4	0.66	0.06	4	1.7

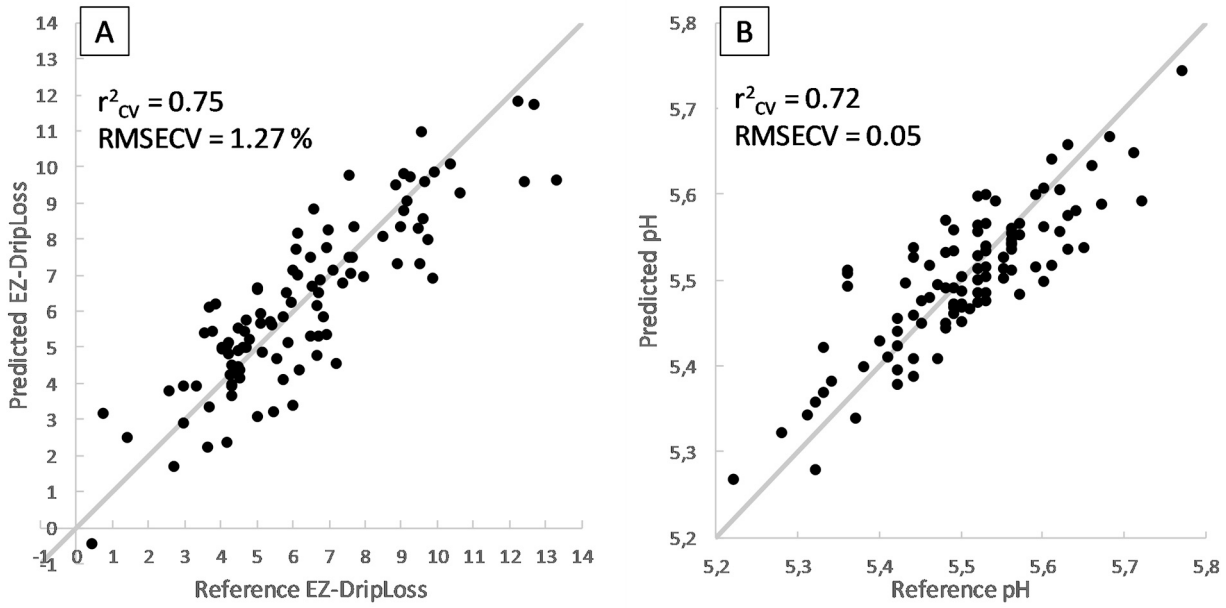

Fig. 3. Predicted vs. reference measurements drip loss (A) and pH (B) from PLSR models of ventral sub-samples using only significant variables from uncertainty tests in the models.

while there were no significant differences in fatty acid signatures (e.g., carbonyl stretch). This indicates that there are locations within the muscle that are better suited for spectroscopic analyses than others.

Inspection of regression coefficients for DL (Fig. 2.C) showed that there were three regions of importance for the model, 810–850 cm^{-1} (peak at 830 cm^{-1}), 936–998 cm^{-1} (peaks at 940 and 995 cm^{-1}) and 1037–1058 cm^{-1} (peak at 1045 cm^{-1}). The most important Raman regions for pH_u were from 510 to 550 cm^{-1} (peaks at 514 and 541 cm^{-1}) and peaks at approx. 830, 900, 940, 986 and 1071 cm^{-1} (Fig. 2.D). Similar regions in the pH_u and DL models had their sign reversed, as expected from the negative correlation between these two parameters. Since the correlation between pH_u and DL was relatively high, it is possible that the model for DL over emphasizes regions related to pH. Thus, it might result in poorer models for DL if the correlation between pH and DL is no longer upheld. Visual comparison between the difference spectrum in Fig. 2.B and the regression coefficients for DL in Fig. 2.C revealed many similarities, e.g. peaks at approx. 830 and 940 cm^{-1}. There were some differences as well, e.g. peak at approx. 1440 cm^{-1}, showing that this peak was not important for prediction of DL as evidenced by the small regression coefficients from the PLSR model.

The region from 510 to 550 cm^{-1} can be assigned to disulfide bonds in cysteine (Sugeta & Go, 1972), which have peaks at 510, 525 and 540 cm^{-1} depending on the conformation of the C-C-S-S-C-C group, and this modality has been shown to be sensitive to changes in pH (Ellepola, Choi, Phillips, & Ma, 2006). The peak at 541 cm^{-1} can also be assigned to lactate (Scheier, Kohler, & Schmidt, 2014), which is in correspondence with the negative sign of this peak for the regression coefficients for the pH_u model, meaning that a higher concentration of lactate results in lower estimates of pH_u. The region around 830 cm^{-1} can be assigned to tyrosine, which has been shown to exhibit pH-related changes in Raman spectra (Xie, Jiang, & Ben-Amotz, 2005), but changes in this region can also be attributed to creatine, which has peaks at 605, 826 and 1045 cm^{-1} (Scheier et al., 2014b). Both lactate and creatine might contribute to the peak at approx. 1040 cm^{-1}, and are expected to increase in intensity in samples with low pH_u. The region around 940 cm^{-1} can be assigned to α-helical secondary structure in muscle proteins (Pezolet, Pigeongosselin, Nadeau, & Caille, 1980) or glycogen content in the muscle, and both have shown decrease in Raman intensity when pH decreased (Andersen, Veiseth-Kent, & Wold, 2017; Scheier et al., 2014b). Bands at approx. 980 and 1080 cm^{-1} are assigned to monobasic and dibasic phosphate groups (Xie et al., 2005), which have been shown to be good probes for determination of pH (Scheier & Schmidt, 2013).

Both the r^2_{CV} and RMSECV from the PLSR models are close in value to the calculated max r^2 and SER, meaning that there is not much room for improvement of the models, assuming that the estimated values were close to the true range for pH and DL. Another aspect was the use of a high-volume Raman probe with a laser spot size of 6 mm, assuring that a sizeable volume of each sample was analyzed. Taken together, this might be as good as it gets when making models from relatively imprecise reference measurement such as DL and to some extent pH.

There are however some challenges related to commercial implementation of the results from the current study. Firstly, the pigs used for analysis are known to be less diverse than what typically gets processed at abattoirs, e.g., only boars of a certain age were included and they are

known to have low intramuscular fat content (Andersen et al., 2018), meaning that the use of Raman spectroscopy for assessment of DL and pH needs to be validated further by incorporating samples with a larger span in origin and relevant meat attributes. Secondly, these measurements were conducted four to five days postmortem, and it is not certain that new models based on spectra recorded at earlier stages, e.g. immediately after slaughter or at day one postmortem, will give similar results as those reported in the current study. To clarify, there are many postmortem processes taking place in meat the first hours and days after slaughter, meaning that a Raman spectrum collected at an earlier time is different from one collected four days postmortem (Scheier et al., 2014a; Scheier et al., 2014b; Scheier et al., 2015). In addition, these postmortem processes can complicate the modelling of DL at an early stage because the meat is still undergoing significant physical and chemical changes. For implementation purposes this needs to be investigated on a case-by-case basis, establishing when it is most appropriate to conduct Raman analysis and the performance of the method in the actual case.

4. Conclusions

Collecting data from the same location with both Raman spectroscopy and DL gave a slight improvement in relative ability to predict drip loss compared to earlier studies. The ventral part of the *LL* slice was best suited for making PLSR models, both for DL and pH_u. Which location to analyze within the muscle should be considered when designing new studies or guidelines for spectroscopy and meat quality. The errors for PLSR models were close to the error of the reference measurements, meaning that improvements to performance of existing models may be difficult to achieve. Overall, results from this study indicate that Raman spectroscopy can be used for rough screening of WHC and pH_u in pork.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2020.108357.

Declaration of Competing Interest

None

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References

Andersen, P. V., Veiseth-Kent, E., & Wold, J. P. (2017). Analyzing pH-induced changes in a myofibril model system with vibrational and fluorescence spectroscopy. *Meat Science, 125*, 1–9.

Andersen, P. V., Wold, J. P., Gjerlaug-Enger, E., & Veiseth-Kent, E. (2018). Predicting post-mortem meat quality in porcine longissimus lumborum using Raman, near infrared and fluorescence spectroscopy. *Meat Science, 145*, 94–100.

Barnes, R. J., Dhanoa, M. S., & Lister, S. J. (1989). Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Applied Spectroscopy, 43*(5), 772–777.

Beganovic, A., Hawthorne, L. M., Bach, K., & Huck, C. W. (2019). Critical review on the utilization of handheld and portable Raman spectrometry in meat science. *Foods, 8*(2), 1–18.

Dardenne, P. (2010). Some considerations about NIR spectroscopy: Closing speech at NIR-2009. *NIR news, 21*(1), 8–14.

Ellepola, S. W., Choi, S. M., Phillips, D. L., & Ma, C. Y. (2006). Raman spectroscopic study of rice globulin. *Journal of Cereal Science, 43*(1), 85–93.

Gjerlaug-Enger, E., Aass, L., Odegard, J., & Vangen, O. (2010). Genetic parameters of meat quality traits in two pig breeds measured by rapid methods. *Animal, 4*(11), 1832–1843.

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science, 49*(4), 447–457.

Huff-Lonergan, E., Baas, T. J., Malek, M., Dekkers, J. C., Prusa, K., & Rothschild, M. F. (2002). Correlations among selected pork quality traits. *Journal of Animal Science, 80*(3), 617–627.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science, 71*(1), 194–204.

Hughes, J. M., Oiseth, S. K., Purslow, P. P., & Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science, 98*(3), 520–532.

Lawrie, R. A. (1985). *Chapter 10 - The eating quality of meat Meat Science (Fourth Edition) (pp. 169–207): Pergamon*.

Li-Chan, E. C. Y. (1996). The applications of Raman spectroscopy in food science. *Trends in Food Science & Technology, 7*(11), 361–370.

Lieber, C. A., & Mahadevan-Jansen, A. (2003). Automated method for subtraction of fluorescence from biological Raman spectra. *Applied Spectroscopy, 57*(11), 1363–1367.

Mark, H., & Workman, J. (2003). *Statistics in spectroscopy*. Elsevier Science.

Martens, H., & Martens, M. (2000). Modified Jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). *Food Quality and Preference, 11*(1–2), 5–16.

Ostovar Pour, S., Fowler, S. M., Hopkins, D. L., Torley, P. J., Gill, H., & Blanch, E. W. (2019). Investigation of chemical composition of meat using spatially off-set Raman spectroscopy. *Analyst, 144*(8), 2618–2627.

Pedersen, D. K., Morel, S., Andersen, H. J., & Balling Engelsen, S. (2003). Early prediction of water-holding capacity in meat by multivariate vibrational spectroscopy. *Meat Science, 65*(1), 581–592.

Pezolet, M., Pigeongosselin, M., Nadeau, J., & Caille, J. P. (1980). Laser Raman-scattering - a molecular probe of the contractile state of intact single muscle-Fibers. *Biophysical Journal, 31*(1), 1–8.

Rasmussen, A. J., & Andersson, M. (1996). *(1–6 September). New method for determination of drip loss in pork muscles. Paper presented at the In Proceedings 42nd international congress of meat science and technology, Lillehammer, Norway*.

Scheier, R., Bauer, A., & Schmidt, H. (2014). Early postmortem prediction of meat quality traits of porcine semimembranosus muscles using a portable Raman system. *Food and Bioprocess Technology, 7*(9), 2732–2741.

Scheier, R., Kohler, J., & Schmidt, H. (2014). Identification of the early postmortem metabolic state of porcine M. semimembranosus using Raman spectroscopy. *Vibrational Spectroscopy, 70*, 12–17.

Scheier, R., Scheeder, M., & Schmidt, H. (2015). Prediction of pork quality at the slaughter line using a portable Raman device. *Meat Science, 103*, 96–103.

Scheier, R., & Schmidt, H. (2013). Measurement of the pH value in pork meat early postmortem by Raman spectroscopy. *Applied Physics B-Lasers and Optics, 111*(2), 289–297.

Schmidt, H., Sowoidnich, K., & Kronfeldt, H. D. (2010). A prototype hand-held raman sensor for the in situ characterization of meat quality. *Applied Spectroscopy, 64*(8), 888–894.

Sugeta, H., & Go, A. (1972). S-S and C-S Stretching Vibrations and Molecular Conformations of Dialkyl Disulfides and Cystine. *Chemistry Letters*(1), 83−+.

Torley, P. J., D'Arcy, B. R., & Trout, G. R. (2000). The effect of ionic strength, polyphosphates type, pH, cooking temperature and preblending on the functional properties of normal and pale, soft, exudative (PSE) pork. *Meat Science, 55*(4), 451–462.

Warriss, P. D., & Brown, S. N. (1987). The relationships between initial pH, reflectance and exudation in pig muscle. *Meat Science, 20*(1), 65–74.

Williams, P. (2014). The RPD statistic: A tutorial note. *NIR news, 25*(1), 22–26.

Williams, P. C., & Sobering, D. C. (1993). Comparison of commercial near infrared transmittance and reflectance instruments for analysis of whole grains and seeds. *Journal of Near Infrared Spectroscopy, 1*(1), 25–32.

Xie, Y., Jiang, Y., & Ben-Amotz, D. (2005). Detection of amino acid and peptide phosphate protonation using Raman spectroscopy. *Analytical Biochemistry, 343*(2), 223–230.