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Predicting post-mortem meat quality in porcine *longissimus lumborum* using Raman, near infrared and fluorescence spectroscopy

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ABSTRACT

Spectroscopic techniques can provide valuable information about post-mortem meat quality. In the current study, Raman, NIR and fluorescence spectroscopy was used to analyze pH, drip loss and intramuscular fat in pork *longissimus lumborum* (n = 122) at 4–5 days post-mortem. Results were promising for partial least squares regression (PLSR) from Raman spectroscopy, giving coefficients of determination from cross validation (r_{cv}^2) ranging from 0.49 to 0.73 for all attributes examined. Important regions in the PLSR models from Raman spectroscopy were attributed to changes in concentrations of post-mortem metabolites and modifications of protein secondary structure. Near infrared and fluorescence spectroscopy showed limited ability to analyze quality, with r_{cv}^2 ranging from 0.06 to 0.57 and 0.04 to 0.18, respectively. This study encourages further research on the subject of Raman spectroscopy as a technique for meat quality analysis.

1. Introduction

One of the most important quality parameters for pork is water-holding capacity (WHC), affecting monetary value, processing properties (Torley, D'Arcy, & Trout, 2000) and eating quality (Hughes, Oiseth, Purslow, & Warner, 2014). Many factors influence WHC of meat, including rate of post-mortem pH decline and ultimate pH (pH_u) (Warriss & Brown, 1987), proteolysis (Huff-Lonergan & Lonergan, 2005) and chemical composition of meat (e.g. intramuscular fat (IMF)) (Lawrie, 1985), illustrating the complexity of this property. WHC of fresh meat is usually measured as amount of drip formed from an intact meat sample, e.g. the bag method (Honikel, 1998) and EZ-DripLoss method (Rasmussen & Andersson, 1996), which are invasive, labor- and time-consuming methods. Even the standard method for measuring pH requires a glass probe to be inserted into the meat and manually recording the pH-value. Development of rapid and non-invasive methods for meat quality assessment for on-line or at-line application is consequently of interest to the meat industry, for amongst others meat classification and optimization of production procedures. To this end, there have been many studies conducted utilizing spectroscopic techniques to analyze pH, WHC and chemical composition of meat. The most promising techniques for implementation in the abattoir are near infrared (NIR), Raman and fluorescence spectroscopy, because they are all non-invasive and rapid techniques that can be implemented in an abattoir.

NIR spectroscopy has great potential for meat quality analysis because the technique measures absorption corresponding to overtones and combinations of vibrational modes involving C—H, O—H and N—H chemical bonds, which in principle makes it possible to analyze composition and functional properties of meat (Osborne, 2006). The use of NIR spectroscopy for meat analysis has been thoroughly reviewed within the last decade, showing the substantial effort put forth in this field (Prieto, Pawluczyk, Dugan, & Aalhus, 2017; Prieto, Roehe, Lavin, Batten, & Andres, 2009; Weeranantanaphan, Downey, Allen, & Sun, 2011). To the best of our knowledge, the benchmark of performance for VIS-NIR spectroscopy performed on pork are as follows: pH: coefficient of determination (r_{cv}^2) = 0.82 and root mean square error of cross validation (RMSECV) = 0.10 (Liao, Fan, & Cheng, 2010); drip loss: r_p^2 = 0.76 and root mean square error of prediction (RMSEP) = 0.8% (Kapper, Klont, Verdonk, Williams, & Urlings, 2012); and IMF: r_{cv}^2 = 0.96 and RMSECV = 0.46% (Prevolnik et al., 2005). Although many studies have shown great promise, no NIR instruments for commercial use for prediction of pH and WHC have been developed.

Raman spectroscopy can provide information about proteins, such as peptide backbone structure and amino acid side-chain properties, as well as characterization of fat, making it a suitable technique for analysis of meat quality (Li-Chan, 1996). Raman spectroscopy was first used for analysis of WHC in pork in 2003 and the results were very

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promising (r_{cv}^2 = 0.98 and RMSECV = 0.27), but the sample size was small (n = 14) and the authors cited a need for further attention in future studies (Pedersen, Morel, Andersen, & Balling Engelsen, 2003). Raman spectroscopy has gained some traction for pork quality analysis in the last few years with the development of a handheld Raman instrument (Schmidt, Sowoidnich, & Kronfeldt, 2010). Results of pH_u and drip loss predictions have been promising from Raman spectra acquired between 30 and 120 min post-mortem in the abattoir, being able to predict pH_u with r_{cv}^2 = 0.68 and RMSECV = 0.09 and drip loss with r_{cv}^2 = 0.73 and RMSECV = 1.0% in one study (Scheier, Bauer, & Schmidt, 2014), and pH with r_{cv}^2 = 0.31 and RMSECV = 0.05 and drip loss with r_{cv}^2 = 0.52 and RMSECV = 0.6% in a follow-up study (Scheier, Scheeder, & Schmidt, 2015). We are unaware of any studies using Raman spectroscopy to analyze IMF of intact pork, but a study has been conducted for lamb meat, resulting in a r_{cv}^2 = 0.02 and RMSECV = 1.2% for IMF (Fowler, Ponnampalam, Schmidt, Wynn, & Hopkins, 2015).

Not many studies have been conducted using fluorescence spectroscopy to analyze fresh pork quality. One of the few studies analyzing fresh pork quality with fluorescence was carried out by Brondum et al. (2000), where drip loss was predicted with r^2 = 0.68 and SEP = 2.27% and IMF was predicted with r^2 = 0.57 and SEP = 1.09%. Fluorescence spectroscopy has also shown promise to analyze pH in a model system containing isolated myofibrils from pork (Andersen, Veiseth-Kent, & Wold, 2017), encouraging further research in this area.

The main aim of this work was to investigate the potential for Raman, NIR and fluorescence spectroscopy to predict drip loss and measure pH_u of fresh pork, with a secondary aim to measure IMF. Using three spectroscopic techniques on the same set of samples allows for comparison of spectroscopic techniques under similar conditions, possibly indicating which techniques should be the focus in future research.

2. Materials and methods

2.1. Animals and meat quality analyses

A selection of 122 Norwegian Landrace boars from an ongoing testing program at Norsvin's boar test station in southeastern Norway were part of this study. The boars were fed ad libitum on conventional concentrates, and the average start and end weight at the test station was 35 to 120 kg live weight, respectively. The boars were slaughtered in eight batches at a commercial abattoir over a period of 9 months. The animals were stunned with 90% CO_2, followed by exsanguination, scalding and splitting within 30 min post-mortem. After 45 min the carcasses were transported through a cooling tunnel (−22 °C, air velocity 8–10 m/s). Following this, the carcasses were chilled in a cooler at 1 °C to 3 °C for 20 h until a core temperature of 7 °C was reached. Finally, the carcasses were transported to a partial dissection line at Animalia, the Norwegian Meat and Poultry Research Centre.

At 4 or 5 days postmortem, the loin muscle (LL – *Longissimus lumborum*) was dissected from the right side of the carcasses, trimmed for fat and used for assessment of multiple meat quality traits and spectroscopic measurements as described in the following. Ultimate pH was measured at the last rib curvature using an insertion pH electrode (WTW 82362, pH 330i, Welheim, Germany). A 5-cm slice of the muscle (positioned 2 cm anterior and 3 cm posterior to the last rib curvature) was homogenized by grinding for 30 s using a mixer (Robot Coupe r5a +, W 1100, Robot Coupe, USA, Inc.) for subsequent measurement of IMF as described by Gjerlaug-Enger, Aass, Odegard, and Vangen (2010).

Assessments of drip loss were performed using two different methods, the EZ-DripLoss method and purge loss in vacuum packages. For the EZ-DripLoss measurement (Rasmussen & Andersson, 1996), two samples at fixed locations on a 2-cm slice (positioned 3 to 5 cm posterior to the last rib curvature) were cut using a circular knife (2.5 cm diameter). Samples were placed in drip loss containers (C. Christensen ApS, Denmark), and stored at 4 °C for 24 h, after which the weight of the drip loss was measured, and expressed as a percentage of the initial sample weight. For the purge loss measurement, a 5-cm thick slice (positioned 8 to 13 cm posterior to the last rib curvature) was weighed before being placed in a plastic bag and vacuum packed using 98% vacuum. The vacuum packed slices were placed in a single layer on a rack in a cooler (4 °C), and stored for 8 days, after which the bags were opened, and the meat gently dabbed with paper before weighing again. Purge was calculated as a percentage of the initial sample weight.

2.2. Spectroscopic analysis

A freshly cut slice of approx. 3 cm (positioned 5 to 8 cm posterior to the last rib curvature) from *LL* was used for spectroscopic analyses at 4–5 days post-mortem. All samples were analyzed with NIR spectroscopy first, followed by fluorescence spectroscopy and finally Raman spectroscopy.

2.2.1. NIR spectroscopy

The meat slice designated for spectroscopy was cut and mounted in a Rapid content module sample cell (FOSS Analytical, Hillerød, Denmark). A spectrum from a sample surface with a diameter of 17.25 mm was recorded at eight different locations on the meat surface using an XDS Rapid content analyzer (FOSS Analytical, Hillerød, Denmark) measuring in the 400–2500 nm wavelength region at 0.5 nm intervals. Spectra were recorded as log(1/R) with FOSS NIRSystem Vision software. All spectra from one sample were averaged prior to further analysis.

2.2.2. Fluorescence spectroscopy

Fluorescence was measured in front face mode on the same sample surface as was measured with NIR. The measurements were carried out with a FluoroMax-4 (Horiba Scientific, Edison, NJ, USA) in front face mode via a FL-300/FM43000 bifurcated fiber-optic probe (Horiba Scientific). The distance between the probe head and sample was about 5 cm and created a circular measurement area of 40 mm diameter. Probe and sample were covered by a black shield to avoid ambient straylight. Emission spectra in the region from 300 to 500 nm (2 nm intervals) were recorded for excitation at 292 nm.

2.2.3. Raman spectroscopy

The sample was cut into three slices and one spectrum was recorded from the freshly cut surface of each slice using a Kaiser RamanRXN2™ Multi-channel Raman analyzer (Kaiser Optical Systems, Inc., Ann Arbor, MI, USA) with a spectral resolution of 5 cm^{-1}. The spectrometer was equipped with a 785 nm laser and PhAT probe, measuring a spot size of 6 mm in diameter. The spectra were recorded with a laser power set to 400 mW in the range of 150–1890 cm^{-1} with 0.3 cm^{-1} intervals and exposure of 3 times 15 s was used for acquisition. Instrument set-up and experiment was controlled using iC Raman version X software (Mettler Toledo, Greifensee, Switzerland).

2.3. Pre-processing of spectra and data analysis

2.3.1. Pre-processing of spectra

Pre-processing of spectral data was done to give comparable spectra for further analysis, by reducing or removing the impact of noise, scatter effects and other undesirable alterations in the spectra.

The three Raman spectra from each sample were averaged. The oxygen peak from 1530 to 1570 cm^{-1} was removed from the spectra by cutting out the variables from the spectrum matrix prior to further pre-processing in the range from 450 to 1775 cm^{-1}. Raman spectra were first base-line corrected and fluorescence background was removed using polynomial curve-fitting (Lieber & Mahadevan-Jansen, 2003), before second order extended multiplicative scattering correction was

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