RESEARCHREPORT
PORK QUALITY

Title: PREDICTION OF PORK QUALITY USING RAMAN SPECTROSCOPY - NPB #15-078

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Industry Summary

The objective of the project was to develop an approach to use Raman spectroscopy to predict sensory quality of fresh pork loins. Pork loins (n=200) were selected during fabrication on seven different production days and six different production plants. Loins were selected to represent the range of quality features. Scans of the ventral portion of the intact loin were collected with a portable Raman spectrometer. Scan time was 6 seconds. Loins were then packaged and aged 14 days. Pork chops from each loin were cut and packaged 14 days postmortem. Scans of the cross section of central loin chops were collected on all 200 loins. Each chop was scanned in three locations of the cross section with a scan time of 6 seconds. Pork sensory quality was evaluated on a select group of loins (n=75 per group) to determine sensory tenderness, chewiness, juiciness, flavor, and off-flavor. Slice shear force was measured on all loins (n=200 per group).

Raman peaks are represented by their wavenumber (Raman shift) and intensity. The peak intensities are dependent on many factors that may vary from sample to sample (i.e., sample size, exposure time, etc.), but their Raman shifts (i.e., the peak wavelengths) remain identical as long as the molecular makeup is the same. These data are summarized using a barcode approach to highlight unique Raman shift “fingerprints” of each sample. Binary barcodes are generated for each sample based on the second derivative spectra collected. Each sample has a barcode that is specific to its Raman spectral properties. The barcode was used to determine the association with sensory traits and pH traits.

Raman spectral properties of pork loin at day 1 postmortem were approximately 60-70 % accurate in predicting aged pork loin tenderness traits. Raman properties of each pork loin after aging were generally over 90 % accurate. Prediction of cooked pork loin juiciness was better than that for tenderness traits.

Aged pork pH was accurately predicted with a regression curve of 11 Raman peaks (within 0.17 pH units). Inclusion of all the spectral data resulted in an improvement in the accuracy of pH prediction to within 0.05 units. This finding is significant and shows promise of developing a non-invasive, non-destructive method to measure an important fresh pork quality trait- pH.

Raman spectral properties show promise in prediction of fresh pork quality. Adoption of the procedure will require engineering to allow measurement at line speeds.

Key words: Raman spectroscopy, Pork loin, pH, tenderness, instrument grading

Introduction

Investigations to determine the major contributors to meat tenderness have been conducted for many years. This is testimony to the persistence of the problem and to the elusiveness of a sustainable solution to the problem. Meat processors, chefs, and retailers all recognize that pork tenderness is a very important quality trait for their customers. Clearly, descriptors on labels and menus often use some verbiage to share that the pork item is tender. Postmortem processing has given processors several approaches to improve tenderness. What is needed is the ability to predict tenderness of pork with a rapid test, preferably early in the production chain. A practical approach is a method that can identify product that is not tender or will not become tender. Removal of pork loins that are not tender will have the favorable result of improving tenderness and decreasing the variability of tenderness of the remaining pork. Product identified as being inferior could then be targeted for a tenderness intervention to improve the product quality. Similarly, identification of pork loins that are of superior tenderness would allow producers, processors, and chefs to confidently market a premium product. **Therefore, the goal of the summarized work was to develop methods to rapidly identify pork loins that are of superior quality AND pork loins that are of inferior quality, with a specific focus on tenderness**.

There is a great need for a rapid, nondestructive analysis technique that can be used to predict consumer response to a pork product. This is especially true for on-line techniques that can be incorporated into the meat processing line. A wide range of physical and chemical methods have been developed and tested for this purpose, including various shearing techniques, pH value, image texture analysis, UV fluorescence, NIR, and ultrasound. Apart from shear force measurements, none of the above methods have found wide applications in the meat industry, mainly due to poor reproducibility and inconsistent correlation to sensory panel evaluations. **Raman spectroscopy** is a technique that has not been fully explored in meat quality assay. Sharing with other vibrational spectroscopic techniques such as Near Infrared spectroscopy (NIR) and Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy yields fingerprints of chemical functional groups that are directly correlated to chemical and physical properties of the pork samples, which ultimately determine the quality of the meat. *Unlike NIR and FT-IR, Raman spectroscopy is insensitive to water, which is a major component of meat and poses as significant background interference in NIR and FT-IR measurements*. This approach thus has the potential to overcome some of the pitfalls preventing other methods from being widely adopted. In addition, Raman spectrosensing requires no sample preparation, and is nondestructive. It is possible to incorporate it into on-line meat processing to provide real-time, continuous monitoring of meat products. Raman spectra reveal the chemical and structural makeup of meat, which have strong correlation to the sensory attributes (e.g., tenderness) of meat.

Our objectives in this project are to 1) integrate Raman spectrosensing with mathematical predictive models to provide objective, rapid and reliable evaluation of meat quality that is consistent with sensory panel assessments; 2) to establish the correlation between temporal changes in meat composition and structure, Raman spectroscopic signatures of meat, and pork sensory quality to further enhance the predictive capability of these powerful techniques. Our rationale is that success in advancing this much-needed technology will expand our knowledge base on how to effectively and efficiently characterize the chemical and physical properties of pork samples, and how to relate subjective sensory attributes of pork samples to objective spectroscopic signatures.

Materials and Methods

Pork loins (n=200) were selected during fabrication on seven different production days and six different production plants. Loins were selected to represent the range of quality features. Scans of the ventral portion of the intact loin were collected with a portable Raman spectrometer. Scan time was 6 seconds. Loins were then packaged and aged 14 days. Pork chops from each loin were cut and packaged 14 days postmortem. Scans of the cross section of central loin chops were collected on all 200 loins. Each chop was scanned in three locations of the cross section with a scan time of 6 seconds. Pork sensory quality was determined on pork loin chops aged

14-16 days as described by Carlson et al., 2017. Slice shear force was determined on pork loin chops aged 14 d (Wheeler, 2005).

Raman spectral data collection at 1 d postmortem in the plant

After the selection, the loins were brought to a reserved area and the ventral side was scanned with the Raman portable system. The Raman spectrometer (iRaman, B&W Tek, Newark, DE) used in this study was equipped with a 780 nm laser, with a maximum power output of 60 mW. The excitation and Raman scattered photons were collected with a fiber optical probe. According to previous tests, the place for scanning was defined in the lean surface around the 10th rib with a scan time of 6 seconds.

Raman spectral data collection at 14 d postmortem in ISU laboratory

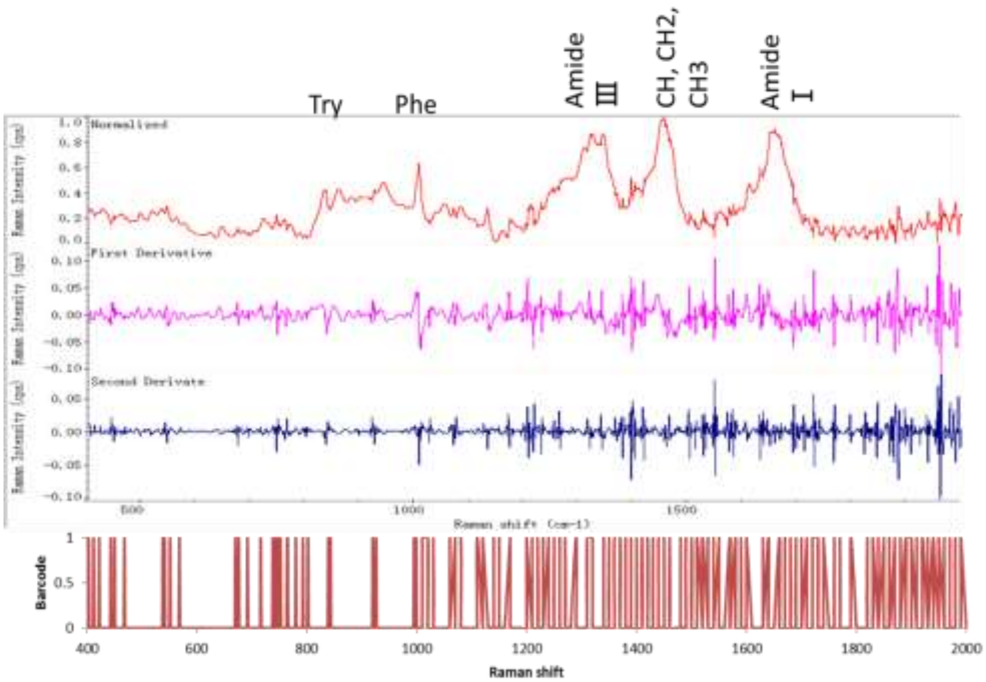
Prior to the scans, the chops were taken off from the package and allowed to bloom for at least 20 minutes. Raman measurements were performed using the same iRaman portable system with the same setting at 3 different locations, with 6 seconds scanning time at each one, inside a dark chamber to reduce the interference from ambient light. The average of the three measurements was used to represent the Raman spectra for each sample. It should be noted that the Raman spectra from the 14 d postmortem samples were acquired from the cross-section of the loins (i.e., chops), not the ventral side surfaces of the loins as in the 1 d postmortem samples. Some of the differences we observed in the spectral patterns may be partially due to these sampling discrepancies.

Spectral data processing

All spectra were automatic baseline corrected and smoothed to reduce the baseline variability at the region between 400 cm^{-1} to 2000 cm^{-1}, and normalized using BWSpec Software Suite (B&W Tek, Inc., Newark, DE). The first derivative and second derivative spectra were calculated from the smoothed and normalized spectra, using Matlab (MathWorks Inc., Natick, MA) (Figure 1).

When Raman spectral data are used to construct chemometric models to classify and/or differentiate pork samples with distinct properties, the most important spectral signatures are the fingerprinting Raman peaks that represent the biochemical landscape of the pork samples. Raman peaks are represented by their wavenumber (Raman shift) and intensity. The peak intensities are dependent on many factors that may vary from sample to sample (i.e., sample size, exposure time, etc.), but their Raman shifts (i.e., the peak wavelengths) remain identical as long as the molecular makeup is the same. Therefore, in this study we utilized a binary barcode approach to eliminate variations in the spectral data due to peak intensities, and highlight the unique Raman shift fingerprints of each sample. The binary barcode approach was originally proposed by Ziegler and coworkers (Martens and Naes, 1992) to differentiate microorganisms based on their Raman spectroscopic signatures. In our previous work we developed a similar approach to improve the classification accuracy of pork loins (Wang et al., 2012). Briefly, binary barcodes were generated based on the second derivative spectra in the 400 cm^{-1} to 2000 cm^{-1} range. A binary value (0 or 1) was assigned to each second derivative spectral data point primarily based on the sigh of the second derivative, i.e., 1 for upward curvature (positive second derivatives), and 0 for downward curvature (negative second derivatives). Furthermore, a threshold for zero was set at 6% of the maximum value of the second derivative for positive second derivative readings (for all value larger than the threshold, 1 was retained; otherwise it was switched to 0). This threshold helps discriminate against residual noise components. Contribution to the measured spectra from low level background noises was thus removed by assigning 0 to it. Remaining 1s represent contributions to the measured spectra from meat components. The threshold value (6%) was determined experimentally by finding the barcodes that provided the best prediction for the sensory attributes. The barcode data were used in classification of the pork samples into different quality groups.

Figure 1. Typical Raman scan of fresh pork loin and depiction of generation of the barcode to represent the data.



Principal component analysis and least square regression modeling

In Raman spectra, each wavenumber represents a dimension or variable. Commonly, data in one Raman spectrum contains thousands of dimensions, which makes statistical analysis much more challenging. For discriminant analysis, as the (number of) dimensions of the data becomes large, the limitation on the capability of detecting distinguishable classes becomes severe (Jimenez and Landgrebe 1998). Due to the fact that most statistical methods are based on optimization criteria, it is advisable to reduce the dimension(s) of the problem, which results in decreasing computational costs and increasing probability of finding the best model representing the data. For this purpose, often a Principal component regression (PCA) is utilized.

$$\mathrm{Y} = \mathrm{L} \times \mathrm{S}^{\mathrm{T}} + \mathrm{E}$$

In the equation above, Y is the matrix of spectra, S is the score matrix, L is the matrix of loadings and E is the error matrix. The data are compressed into PC scores.

All spectra were polynomial baseline corrected, smoothed using moving average algorithms and area normalized at the region between 600cm^{-1} to 2000cm^{-1} before principal component regression (PCA) is utilized. 10-40 PCs (account for at least 90% of total variance in the data) were selected from thousands of dimensional hyper-spectral data as inputs for multivariate discriminant classification model.

Once the PCAs were calculated, least square regression model was constructed to correlate the PCAs as variables to predicted variables, including SSF, tenderness and pH values. The predicted values were then compared to measured ones to determine the accuracy of the prediction.

Classification model development

The multivariate data analysis was carried out using Support Vector Machine (Steinwart & Christmann, 2008) implemented with Matlab SVM toolbox. Partial Least Square (PLS) regression was used to compress the data sets (the binary barcodes) and generate inputs for the SVM model. Comparing to the unsupervised principal component analysis (PCA), PLS is a supervised method in which the PLS scores are obtained to maximize the correlations between them and the predictors (e.g., SSF values and tenderness, juiciness and chewiness scores). Our main goal is to predict sensory attributes (e.g., tenderness, juiciness and chewiness) that are at the two ends of the panel evaluation spectrum ("poor" vs. "good"), which are represented by the top and bottom 25% percentiles in this study.

For sensory tenderness, the 525 pork loin samples were divided into 4 groups according to the percentile of values of sensory tenderness or SSF values. 350 samples were randomly selected as the calibration set; among the remaining 175 samples, 100 were again randomly selected as testing set. Different calibration samples were chosen randomly (this means, for every run, a new set of 350 samples were selected) to calculate the average classification accuracy (10 random replication). Chemometric analysis was conducted using Matlab (The MathWorks, Natick, MA) software. It should be noted that due to instrument malfunction, day 1 postmortem spectral data from factory No.4 were missing. Hence, the corresponding data for tenderness and SSF were omitted for the day 1 postmortem classification model.

pH value prediction

The pH value of pork postmortem is another important quality indicator. pH value changes as time elapses, and the change in pH values affect the molecular environment of the meat which can be monitored through changes in relative peaks in Raman spectra of the meat samples. More specifically, peaks associated with lactate (535, 855, 1305, 1350 and 1414 cm^{-1}), creatine (827 cm^{-1}), phosphate (980 and 1078 cm^{-1}), ATP (1578 cm^{-1}), IMP (1552 cm^{-1}) and carbonyl bond (1714 cm^{-1}). A regression model is developed to correlate pH changes to these peak intensities, as follows:

$$pH = A + \sum_{i=1}^{11} a_i \times S(v_i)$$

Where a_i are regression coefficients, $S(\nu_i)$ are normalized Raman intensities of the above mentioned 11 peaks, and A is a regression constant. A and a's are determined by least-square regression to minimize RMSEC

$$RMSEC = \sqrt{\sum_{i=1}^{1121} (pH_{measured} - pH_{calc})^2}$$

In total, pH values of 1121 samples were used to construct this pH prediction model.
Another approach is to use the entire spectrum of each sample for pH prediction. In this approach, instead of using only the 11 peaks, we used the first 20 PCAs calculated from the spectral pool. Again a regression model is constructed to predict pH, which minimizes RMSEC.

Results

Classification based on Sensory Attributes

The distribution of the three sensory attributes studied is shown in Figure 2.

Figure 2. Distribution of sensory tenderness, chewiness and juiciness

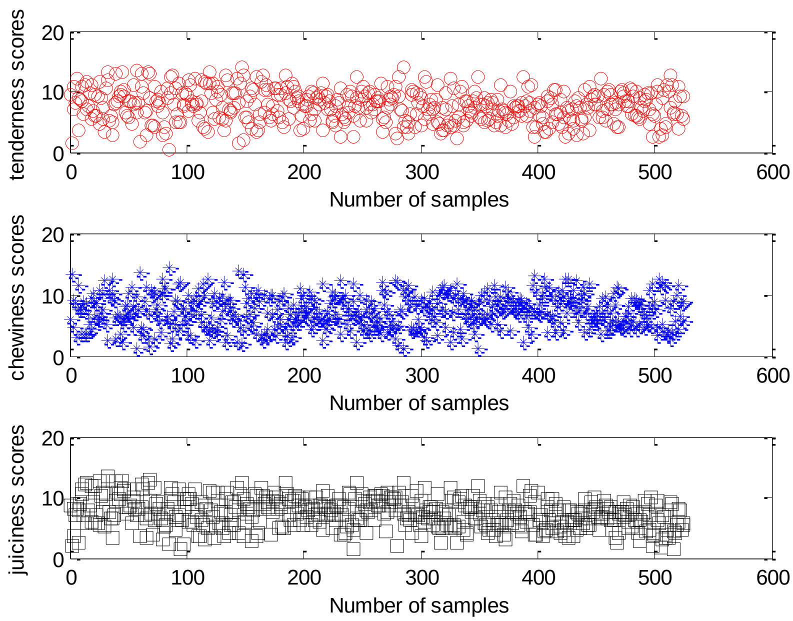


Figure 3. Correlation of Slice Shear force with sensory scores.

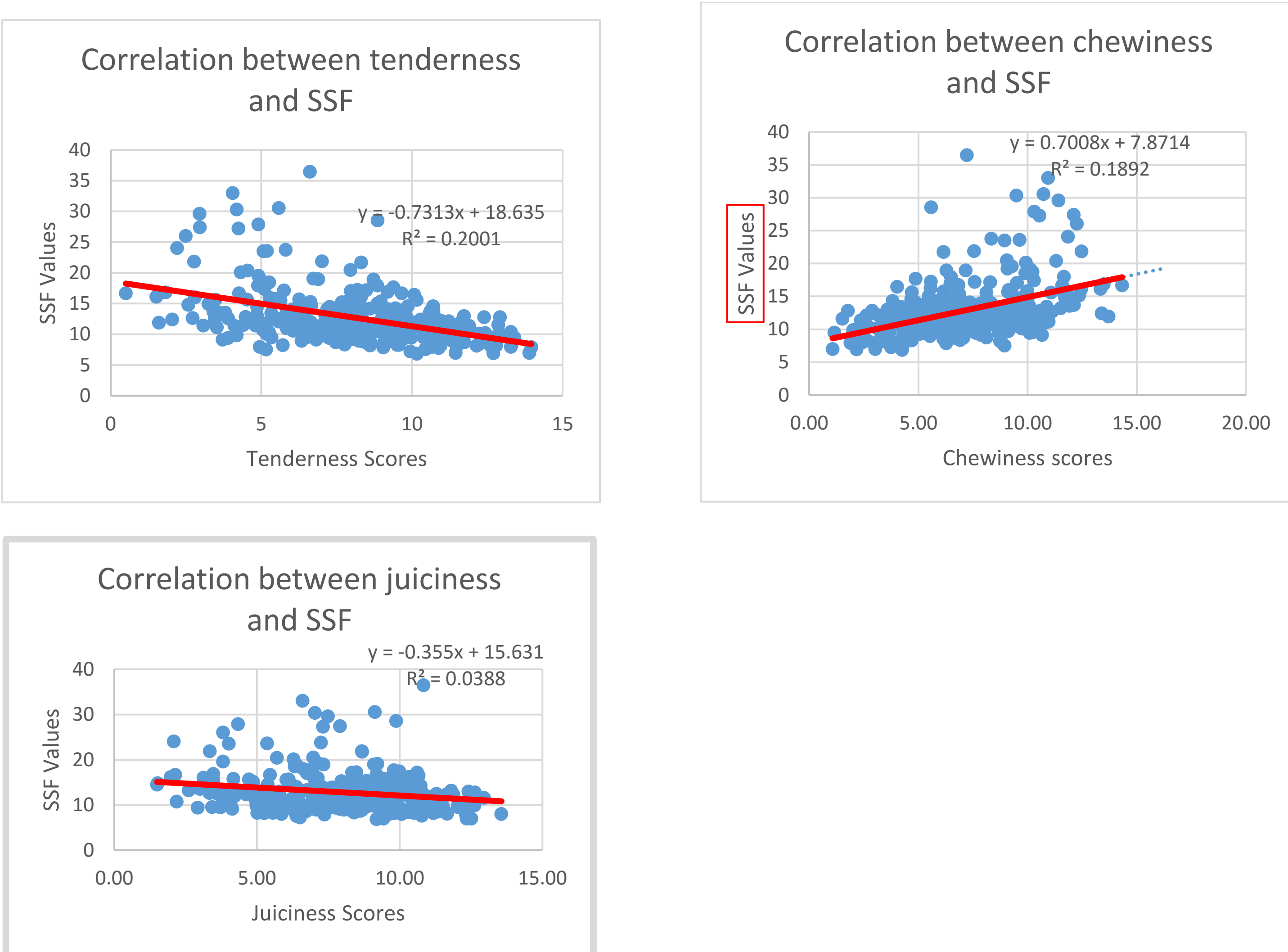


Fig. 3a-c showed the correlation between SSF values and sensory attributes. It appears that only very weak correlations between these parameters could be established.

With 525 samples, classification models based on day 1 and day 14 Raman spectral data were constructed to classify pork samples into 4 25% percentiles. The results are listed in table 1.

Table 1. Classification Results for Tenderness, with 525 samples. Prediction accuracy are average over 10 repetitions.

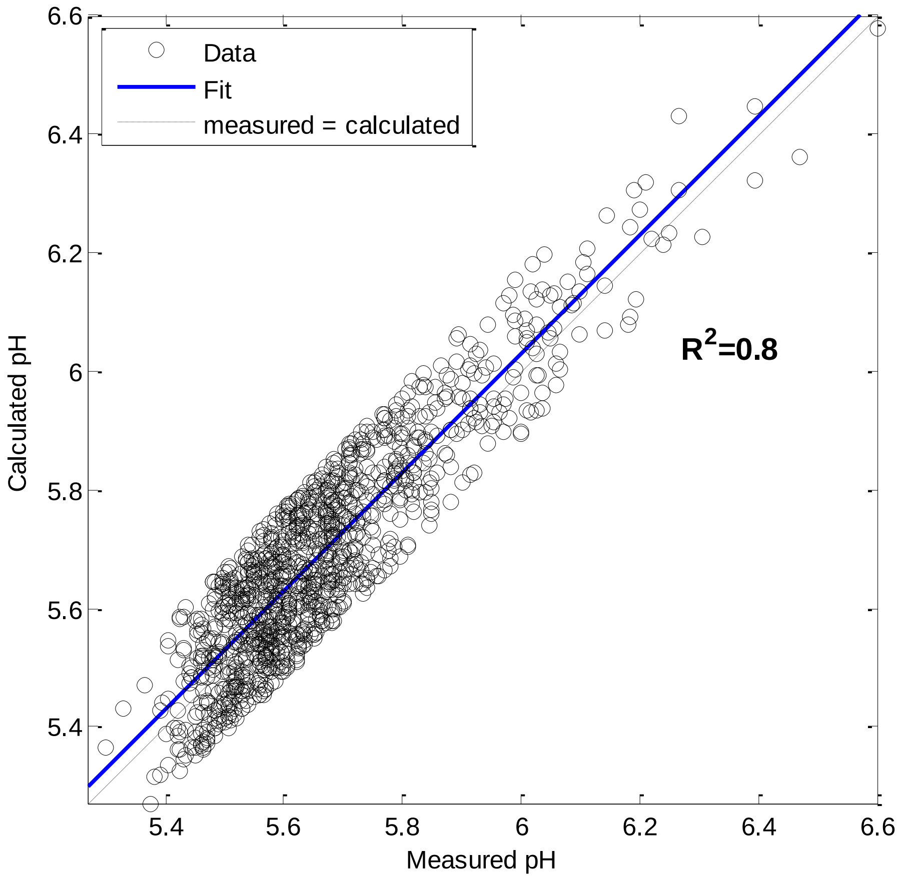
Grouping	1^{st} 25% percentile	2^{nd} 25% percentile	3^{rd} 25% percentile	4^{th} 25% percentile
Day 1 postmortem Tenderness	72.3% ± 4.6%	64.5% ± 4.4%	63.7% ± 4.9%	64.6% ± 5.8%
Day 14 postmortem Tenderness	92.6% ± 3.7%	89.4% ± 3.1%	90.2% ± 6.7%	93.5% ± 4.8%
Day 1 postmortem SSF	78.1% ± 7.2%	74.5% ±2.6%	72.6% ±3.1%	69.9% ± 4.2%
Day 14 postmortem SSF	92.8% ± 3.7%	84.1% ± 7.2%	91.7% ± 5.5%	95.5% ± 3.8%
Day 1 postmortem Chewiness	73.3% ± 3.6%	68.3% ± 5.1%	66..3% ± 4.5%	70.3% ± 5.6%
Day 14 postmortem Chewiness	92.3% ± 4.6%	90.3% ± 2.1%	88.7% ± 2.6%	94.0% ± 2.7%
Day 1 postmortem Juiciness	82.3% ± 1.6%	77.5% ± 2.5%	79.3% ± 5.2%	77.8% ± 0.8%
Day 14 postmortem Juiciness	94.3% ± 2.6%	88.3% ± 4.6%	90.5.3% ± 3.5%	98.5% ± 0.5%

Consistently, day 14 spectral data offer better prediction accuracy. It is reasoned that this is due to the fact that the sensory attributes as well as SSF are all valued at day 14. Spectral signatures of the sample changes as the meat age, hence the day 14 data in general are better indicators of the state of the meat while their sensory attributes are being evaluated.

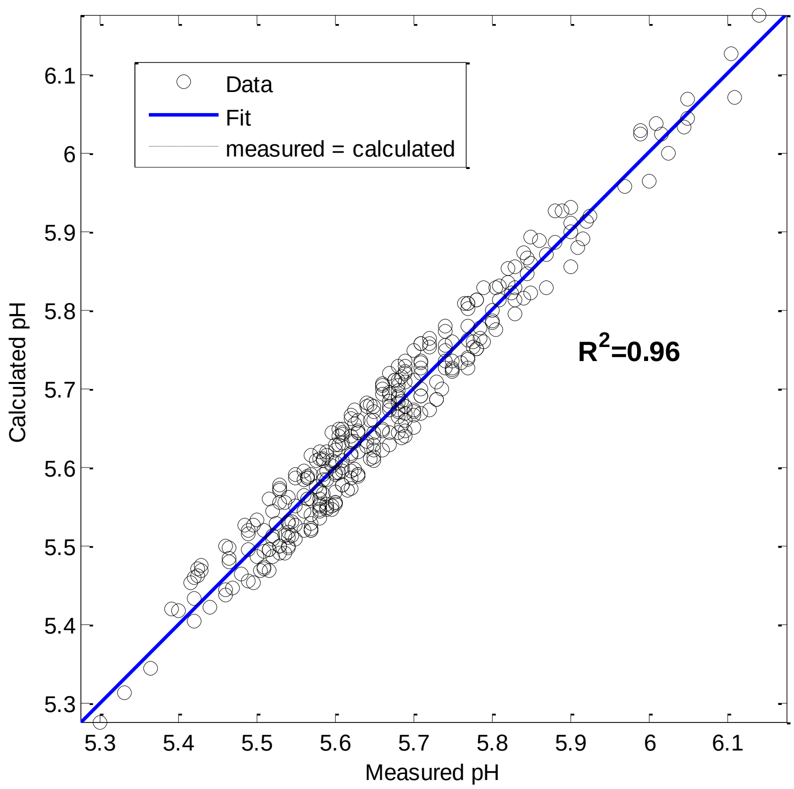
Also interestingly, the prediction of juiciness is better statistically than that for tenderness and chewiness, especially for day 1 postmortem. Reasons for that remain to be unveiled.

pH values for the meat samples day 1 postmortem were used to construct regression models based on both specific peaks (11 in all) and the entire spectra. The regression models are shown in Figure 4 a and b.

Figure 4. Regression of Raman data and fresh pork loin pH.



(a) Regression model with 11 Raman peaks



Regression model with 20 PCAs calculated from entire spectra

With 11 Raman peaks, the regression model yields prediction on pH values within 0.17 pH unit of measured pH, while with the entire spectra the accuracy of the pH prediction is improved to within 0.05 pH unit. Given the fact that the Raman measurement was obtained in 6 seconds, it offers a potentially powerful alternative to pH measurement in a non-invasive and non-destructive manner. It should be noted though the spots at where pH values were measured were not the same as the spots at which Raman spectra were acquired, hence the actual discrepancy between measured pH and predicted ones might be larger than what were observed in this study. However, since local variation in pH is a well-known fact, the method developed here is still practically meaningful.

Discussion and Summary

The results demonstrate that the Raman spectral properties can be used to predict the sensory traits and pH of fresh pork. The measurements made on the pork loin (ventral side) on the fabrication floor (1 day postmortem) were not as effective at predicting the sensory quality as the scans of the loins (cross section) after aging 14 d postmortem. The ventral side of the loin is chosen for the early postmortem sample because it is what would be available in the processing plant. The results also suggest that changes in the pork loin during aging – most notably protein degradation and perhaps water migration – could affect the spectral properties. We propose those changes are specifically related to the sensory tenderness and shear force values.

The most important result of the Raman spectra analysis is that it can be used to predict product pH. Pork loin pH is known to be directly associated with water holding capacity, cook loss, and sensory quality. Importantly, Raman spectra could be collected on pork loins on the fabrication floor in a non-invasive approach to measure (or predict) pH. The measurement is robust because the Raman spectra relate to chemical signals related to pH (lactate, creatine, phosphate, IMP, ATP). Therefore, the scanner can be programmed to search for the important peaks in the spectra.

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