



## Predicting aged pork quality using a portable Raman device

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### ABSTRACT

The utility of Raman spectroscopic signatures of fresh pork loin (1 d & 15 d postmortem) in predicting fresh pork tenderness and slice shear force (SSF) was determined. Partial least square models showed that sensory tenderness and SSF are weakly correlated ( $R^2 = 0.2$ ). Raman spectral data were collected in 6 s using a portable Raman spectrometer (RS). A PLS regression model was developed to predict quantitatively the tenderness scores and SSF values from Raman spectral data, with very limited success. It was discovered that the prediction accuracies for day 15 post mortem samples are significantly greater than that for day 1 postmortem samples. Classification models were developed to predict tenderness at two ends of sensory quality as “poor” vs. “good”. The accuracies of classification into different quality categories (1st to 4th percentile) are also greater for the day 15 postmortem samples for sensory tenderness (93.5% vs 76.3%) and SSF (92.8% vs 76.1%). RS has the potential to become a rapid on-line screening tool for the pork producers to quickly select meats with superior quality and/or cull poor quality to meet market demand/expectations.

### 1. Introduction

Pork meat can be classified into quality groups, according to measurements taken in the carcasses like pH 45 min postmortem, pH 24 h postmortem, drip loss, and L\* value (Bendall & Swatland, 1988; Joo, Kauffman, Kim, & Kim, 1995; Kauffman, 1993; van Laack et al., 1994; Warner, Kauffman, & Greaser, 1997) but these methods may not be amenable to line-speed measurement. Trained sensory panel evaluation is considered the best evaluation method to predict tenderness and provides the most accurate prediction of consumer's responses, but, they are costly, laborious and time consuming, so, they cannot be used as a routine quality assurance method in meat production systems. Based on the available methods and with the difficulties/restrictions in using them on-line, it becomes essential to develop a quick and non-invasive method to evaluate pork sensory quality. A wide range of methods have been investigated regarding an early postmortem assessment of meat quality (ElMasry, Suna, & Allen, 2011; Honikel & Fischer, 1977; Hoving-Bolink et al., 2005; Kamruzzaman, ElMasry, Sun, & Allen, 2012; Toldrá & Flores, 2000) but, for on-line applications,

spectroscopic methods seem more appealing. Raman spectroscopy (RS) is an alternative vibrational spectroscopic method that can be used to evaluate structure and composition of food samples. It is non-invasive and can provide in situ information about composition/structure of proteins and lipids (Beattie, Bell, Borggaard, & Moss, 2008; Beattie, Bell, Farmer, Moss, & Patterson, 2004; Fowler, Schmidt, Ven, Wynn, & Hopkins, 2014; Herrero, 2008a, 2008b; Olsen, Rukke, Flåtten, & Isaksson, 2007; Schmidt, Scheier, & Hopkins, 2013). An added advantage of RS is that water molecules are relatively weak Raman scatters, and they don't yield strong interfering signals like NIR and FT-IR do. The objective of this study was to evaluate the correlation between Raman spectral data measured from fresh and aged pork with sensory characteristics as well with slice shear force (SSF), and to develop classification models to allow rapid/accurate prediction of sensory tenderness groupings based on objectively measured RS and SSF data.

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## 2. Materials and methods

### 2.1. Animals and samples collection

In this work, pork from four different plants across the US were studied. These plants were chosen to represent the variation in processing and product quality. Two hundred loins were selected in each plant. The data presented here comprises the Raman scans and slice shear force measurements of 800 loins and sensory evaluations for 300 loins. The pork loins were removed from the carcass at 24 h postmortem and after routine loin boning and trimming, loins were selected based on color and marbling diversity by USMARC (Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE) personnel. After the measurements in the plants, the boneless loins were vacuum packaged and transported on the same day to USMARC where they were held until 14 days postmortem at 0 °C. After aging, the loins were sliced into 2.54 cm chops with a Grasselli NSL 400 portion meat slicer (Grasselli SPA, Albinea, Italy). Chops 5 and 6 were used for slice shear force, chops 7 and 8 for trained sensory analysis and chop 9, for Raman. For the sensory analysis only 75 loins out of the 200 from each plant were chosen, based on the extreme differences in color and marbling. One chop for Raman measurements (to be scanned on day 15 postmortem) and two for trained sensory analysis were vacuum packaged and transported in a cooler on ice blocks to the ISU Lab on day 14 postmortem.

### 2.2. Raman spectral data collection at day 1 postmortem in the plant

After they were selected for the study, the refrigerated loins (0–3 °C) were brought to an off-line, safe area nearby and the ventral side was scanned with the Raman portable system, under ambient light. The Raman spectrometer (iRaman, B&W Tek, Newark, DE) used in this study was equipped with a 785 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 lean was scanned once, on the surface of *Longissimus* muscle, around the 10th rib, perpendicularly to the muscle fibers, with an integration time of 6 s. Spectral acquisition time (integration time, 6 s) was selected to assure a minimum acceptable signal to noise ratio (SNR, > 3) was reached, with as little interruption to the processing line operation as possible during any future implementation. A home-made probe casing was attached to the head of the optical probe to minimize ambient light during spectral acquisition. For each sample, three spectra were acquired.

### 2.3. Raman spectral data collection at day 15 postmortem in ISU laboratory

Prior to the scans, the chops were removed from the package and allowed to bloom for at least 20 min, at room temperature. Raman measurements were performed inside a dark chamber to reduce the interference from ambient light using the same iRaman portable system described above with the same settings at 3 different locations on the surface of the chops, with 6 s scanning time for each location. 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 15 days 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 day 1 postmortem samples and were the average of 3 scans vs 1 scan from day 1 postmortem measurements. Some of the differences we observed in the spectral patterns may be partially due to these sampling differences.

### 2.4. Physical evaluation - Slice Shear Force (SSF)

Slice shear force on all 800 loins was measured according to

(Shackelford, Wheeler, & Koohmaraie, 1999). Two 2.54-cm thick chops were obtained from the 11th rib region of each loin. The following day (i.e., 15 d postmortem), fresh (never frozen) chops were cooked (71 °C) with a belt grill and longissimus slice shear force (SSF) was measured with a single 1-cm-thick, 5-cm-long slice, removed from the lateral end of each chop. SSF measurements were conducted in duplicate. The obtained SSF values were then averaged and that value was used for all analysis.

### 2.5. Sensory evaluation

After cutting, sensory chops (300, 75 loins from each processing facility) were sealed in vacuum package bags and transported on ice to the Iowa State University Sensory Evaluation Laboratory (Ames, IA). The sensory evaluations occurred on the following three days, using two chops per loin. The chops were cooked on clamshell grills (Cuisinart Griddler Deluxe, Model GR-150, Cuisinart, East Windsor, NJ) to an internal temperature of 68 °C. Individual chop temperatures were monitored using thermocouples attached to a digital temperature monitor (Omega Engineering Inc., Stamford, CT). When samples reached 68 °C, they were removed, and the center of the sample was cut into 2.54 cm cubes. Samples were placed in a Styrofoam cup which had a random three-digit blind code and capped with a plastic lid. Blinding codes were used to identify the sample and to ensure that there was no sample bias. A trained sensory panel ( $n = 8$  to 10) evaluated samples for juiciness, tenderness, chewiness, pork flavor, and off-flavor. A 15-unit unanchored scale was used with terms which represented a low degree of each trait on the left end of the line and a high amount of each trait on the right end. Unsalted crackers and water were provided between samples. Sensory data were collected and summarized using a computerized sensory software system (Compusense Inc., release 5.4, Guelph, ON, Canada).

### 2.6. Classification (grouping) of loins

For groupings, we classified the 300 samples with tenderness scores into 4 percentiles. For sensory tenderness scores, the samples that fell into the top 25% (highest tenderness scores, 1st percentile) were classified as “good”, and the bottom 25% (lowest tenderness scores, 4th percentile) were classified as “poor”. The groups of upper-medium and lower-medium were classified for samples fell in the 2nd percentile (50%–75%) and 3rd percentile (25%–50%), respectively. Each group has 75 samples in it. The same principle also was applied to the 300 samples for SSF grouping analysis, as a comparison to the tenderness score analysis. It should be noticed that high SSF values suggest tough meat, which typically are coincident with low sensory tenderness scores. For sensory tenderness, two hundred samples were randomly selected as the calibration set; among the remaining one hundred samples, fifty samples were again randomly selected as a testing set. Samples from different plants were pooled together for data analysis. Hence, the variances associated with different plants were included as part of the intrinsic variances of the samples in the analysis. Ten repetitions were conducted to calculate the average classification accuracy.

In addition, another grouping analysis was carried out for the entire 800 sample set, with SSF values. Among the 800 samples, three groups were defined as “good”, “medium” and “poor”. “Good” was defined as a SSF value < 10; “medium” was defined as a SSF value between 10 and 15; and “poor” was defined as a SSF value higher than 15. For this analysis, 400 randomly selected samples were used for calibration, and 100 randomly selected samples were used for each validation tests. Ten repetitions were conducted to calculate the average classification accuracy.

## 2.7. Spectral data processing

All Raman spectra were automatic baseline corrected and smoothed to reduce the baseline variability at the region between  $400\text{ cm}^{-1}$  to  $2000\text{ cm}^{-1}$ , 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).

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., laser spot size on sample, exposure time, etc.), however, although the Raman shifts (i.e., the peak wavelengths) may also down-shift or up-shift due to changes in molecular structures that lead to changes in bond lengths, these changes are due to internal changes, not external changes such as varying measurement conditions often met in a factory floor, hence they tend to remain more as constant during Raman measurement. 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 & 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, Lonergan, & Yu, 2012). Briefly, binary barcodes were generated based on the second derivative spectra in the  $400\text{ cm}^{-1}$  to  $2000\text{ cm}^{-1}$  range. A binary value (0 or 1) was assigned to each second derivative spectral data point primarily based on the sign 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 values 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 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 barcodes then were subjected to data compression to reduce the dimension of the data set. 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 separation between groupings correlated with the predictors (e.g., SSF values and tenderness groupings). Our main goal is to predict sensory attributes (e.g., tenderness) that are at the two ends of the panel evaluation spectrum (“poor” vs. “good”). In this work, PLS is only used to compress the dimension of the data. To calculate PLS loadings and scores, the spectral barcode data were correlated to a grouping matrix which used “dummy variables” assigned based on the grouping of the spectral data (for tenderness classification dataset, the variables were 0 for good, 1 for upper-medium, 2 for lower-medium, and 3 for poor; for the whole SSF dataset, the variables were 0 for good, 1 for medium and 2 for poor), and the PLS inherently sought loadings that best separated the group centers. The PLS scores calculated this way were optimized to separate those groups (Kemsley, 1998). This compression was conducted using WINDAS, a software package developed by Kemsley (John Wiley & sons, Chichester, UK), and the PLS loadings and scores were output for the classification model development and test. The loadings matrix was used for transforming barcode data from the test set into PLS scores for

classification testing.

## 2.8. Classification and regression model development

The first ten PLS scores generated from the barcodes were employed in a support vector machine (SVM) discriminant model (Steinwart & Christmann, 2008) to classify unknown pork loin samples into different quality categories implemented with Matlab SVM toolbox.

The 10 PLS scores were also used to create a multiple linear regression (MLR) model to calculate sensory tenderness scores and SSF values. 200 samples were randomly selected from the 300 sample set to create the MLR model, and the remaining 100 samples were used for validation of the model (50 randomly selected samples were used in each validation test). The calculated sensory tenderness scores and SSF values were then compared to the measured values with different error tolerance to evaluate how feasible it is to use such a predictive model to calculate these values quantitatively.

All data analysis was conducted using Matlab (The MathWorks, Natick, MA) software.

## 3. Results and discussion

### 3.1. Sensory tenderness and SSF

One of the key factors that determines the accuracy of statistical predictions is the uniformity of the data distribution. Our primary goal is to correctly predict pork samples that fall into the two extreme ends of their sensory texture attributes (e.g., tenderness). However, it was not feasible to find a large group of pork loins that had uniformly distributed sensory tenderness scores across the entire range of 0–15, among the 300 samples available (Fig. 1a). The tenderness scores were relatively evenly distributed within our evaluation range (0–15). SSF data were measured for all 800 samples, and their distribution was shown in Fig. 1b. The last batch of samples (N° 601–800, from plant No.4) showed a broader range of SSF values than the first three batches (from plant No.1–3), where the tenderness scores for the 75 samples from that batch did not show much discrepancy from the other batches.

For the 300 samples that both sensory tenderness scores and SSF values were available, a least square regression model was developed to evaluate the correlation between these two parameters. The results are shown in Fig. 2. With a  $R^2 = 0.20$ , it suggests that SSF and sensory tenderness scores were only weakly correlated, which is inconsistent with earlier reports from other groups that correlations between sensory tenderness and SSF values generally are moderate to high (Emerson, Woerner, & Belk, 2013; King, Wheeler, Shackelford, & Koohmaraie, 2009; Shackelford, Wheeler, & Koohmaraie, 2004).

### 3.2. Raman spectroscopic analysis

Typical Raman spectra of pork samples in the  $400\text{--}2000\text{ cm}^{-1}$  region are shown in Fig. 3. Baseline correction, smoothing and normalization were applied to reduce background noises. The wave number and intensity changes in the Raman bands were indicative of changes in the secondary and tertiary structures and variations in local environments of meat proteins, which in turn determine the characteristics/properties of the meat. The Raman band centered near  $1653\text{ cm}^{-1}$  (Fig. 3) represents amide I band which is an indicator of the overall concentration of proteins (Herrero, 2008a, 2008b). The Raman band centered near  $1250\text{ cm}^{-1}$  represents amide III which is sensitive to secondary and tertiary structures of proteins (Beattie et al., 2008). Band at  $1450\text{ cm}^{-1}$  is assigned to  $\text{CH}_2$  scissor, which decreases with increasing hydrophobicity in molecular environment; Band at  $1003\text{ cm}^{-1}$  is assigned to Phenylalanine ring stretching, which is insensitive to molecular environment. Bands centered around  $900\text{ cm}^{-1}$  and  $1130\text{ cm}^{-1}$  are assigned to stretching modes of C–C and N from lipids and proteins, respectively (Beattie et al., 2008). Combined these

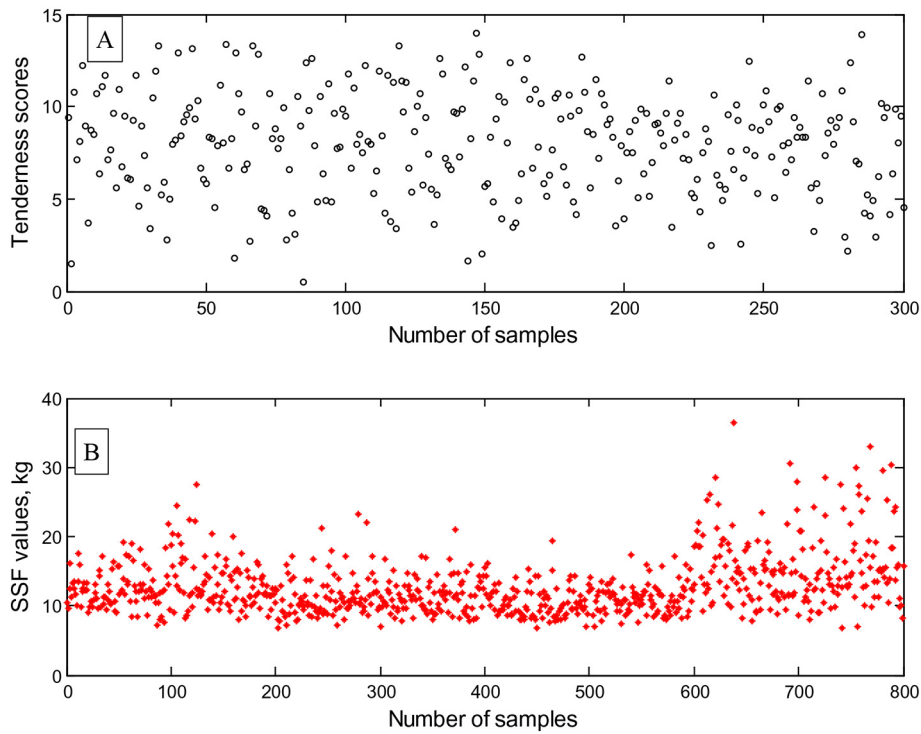


Fig. 1. Distribution of Tenderness scores and Slice Shear Force (SSF) values ( $n = 300$  samples; a. Tenderness scores; b. SSF values).

spectroscopic characteristics illustrate the changing chemical properties of the meat samples.

The Pearson correlation coefficients between sensory tenderness scores, SSF values and Raman intensity at each wavenumber of all spectra of 300 samples at day 1 and day 15 postmortem are shown in Fig. 4a and b. In general, Raman intensities were only lowly ( $< 0.20$ ) correlated to sensory tenderness and SSF values; it is understandable, sensory attributes are complex, nonetheless they cannot always be completely explained by physically measured parameters. SSF values represent the mechanical properties of the meat as a whole, they are also moderately correlated to Raman signatures. Another expected observation was that the correlations between tenderness, SSF and Raman spectral data showed opposite trends. The correlation patterns of tenderness scores and SSF to Raman peaks were similar to what we

have reported before (Wang et al., 2012). This suggests a mechanistic correlation between SSF values and chewiness. Nonetheless, the variations in the correlation readings suggested that the underlining mechanism for tenderness/SSF variations between pork samples might originate from biochemical/compositional variations (e.g., protein composition and structures, proteolysis, lipids content and distribution, etc.).

### 3.3. Prediction of sensory tenderness and SSF values based on PLS regression model

Direct regression modeling using the entire spectral data is very inefficient computationally. Therefore, dimension reduction to the spectral data was conducted before SVM modeling. The first 20 PLS

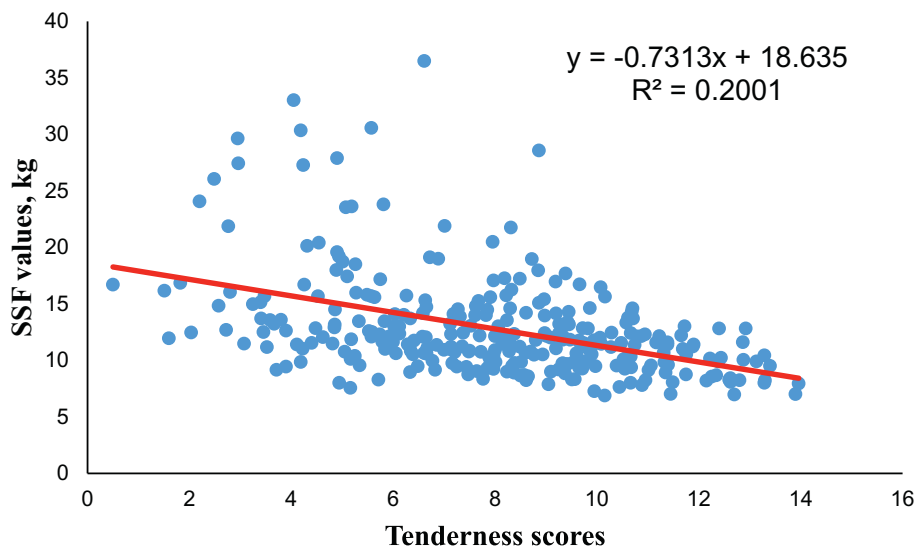


Fig. 2. Linear regressional correlation between Tenderness scores and Slice Shear Force (SSF) values ( $n = 300$  samples).

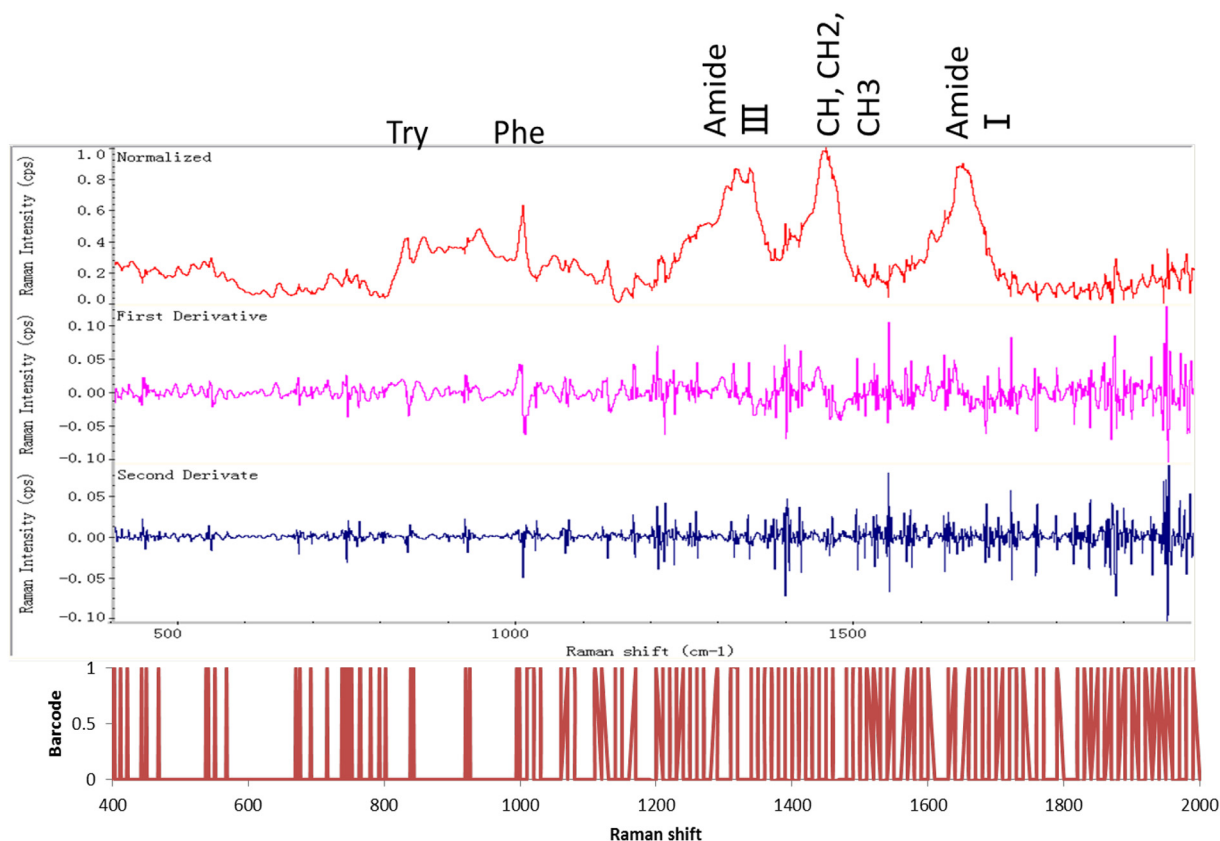


Fig. 3. Typical Raman (iRaman, B&W Tek, Newark, DE) spectrum of pork samples. From top to bottom: Raman spectrum with peak assignment, 1st derivative spectrum, 2nd derivative spectrum, and binary barcode spectrum.

components were calculated from the Raman spectral data, > 95% of the total variances were accounted for by the first 10 PLS components. Also, the first 10 PLS components were well correlated to the attributes (sensory tenderness and SSF) by the calculation of the Pearson correlation coefficients. Hence, the first 10 PLS components were used for regression model development without further optimization.

To develop the MLR model, spectra of 200 pork samples were randomly selected as training set. Within the remaining 100 pork samples, 50 were randomly selected as the validation/testing set. Table 1 shows the validation results. For day 1 postmortem samples, for an error tolerance of 25% (i.e.,  $(1.0 - 0.25) \times \text{observed value} < \text{predicted value} < (1.0 + 0.25) \times \text{observed value}$ ), the prediction accuracies are 50.1% and 30.8% for tenderness scores and SSF values, respectively; for an error tolerance of 50% ( $(1.0 - 0.5) \times \text{observed value} < \text{predicted value} < (1.0 + 0.5) \times \text{observed value}$ ), the prediction accuracies are 61.5% and 57.7%, respectively; for an error tolerance of 100% ( $(1.0 - 1.0) \times \text{observed value} < \text{predicted value} < (1.0 + 1.0) \times \text{observed value}$ ), the prediction accuracy is 92.3% and 88.5%, respectively. It is clear that regression model does not yield accurate prediction of specific values, for either SSF or tenderness scores. Nonetheless, the prediction accuracies for day 15 postmortem samples are significantly higher than that for day 1 postmortem samples, both for tenderness scores and SSF values. For the same 25% error tolerance, the prediction accuracies for tenderness scores and SSF are 75% and 88.5%, respectively. It is understood that postmortem aging influences the biochemical and structural features of muscle and meat components and these were reflected in their spectral characteristics. The data collection sites were different on day 1 (ventral side) and day 15 (cross-section), it might cause some variations in the spectral measurements. However, since the underlining chemical fingerprints ultimately determine the spectral signatures, and the intrinsic chemical differences between these two sites, which are still within close distance

from each other, are fairly insignificant. It is therefore reasoned that these variations should be minimal, comparing to what would be caused by aging. Because both tenderness and SSF were evaluated at day 15 postmortem, it is reasonable to deduce that the spectroscopic signatures of the day 15 postmortem samples were better correlated to them than that of day 1 postmortem samples, which in turn resulted in better prediction results.

The standard deviations of the sensory panel values were around 5%. Apparently, an accurate prediction of either tenderness scores or SSF values based on Raman spectroscopic data is not feasible. Due to the complexity of the mechanism which determines meat tenderness and its mechanical properties, this is to be expected. However, to predict consumer responses to a meat product, it may not be necessary to know the precise sensory panel values, which are subjectively defined. If a prediction can be acquired that distinguishes the extreme cases (i.e., very good quality vs. very poor quality) with good reliability, such prediction would be beneficial to a meat producer to classify its meat products. Therefore, we further developed classification models using Raman spectral data to differentiate and classify pork loins into groups that are defined based on their sensory tenderness scores or SSF values.

#### 3.4. Classification of pork loins by sensory tenderness and SSF

A primary question to answer in this project was to determine if Raman spectra acquired with a portable spectrometer in a short time span (6 s per sample) from freshly cut pork loins could be used to classify pork loins into distinguishable quality grade groups (good, upper-medium, lower-medium, poor) as defined by their tenderness or SSF values. The classification accuracies for each group were presented in Table 2. For day 1 postmortem, the classification was only moderately successful; for both tenderness scores and SSF values, ~70% correct classification was achieved for each group. Such accuracy was

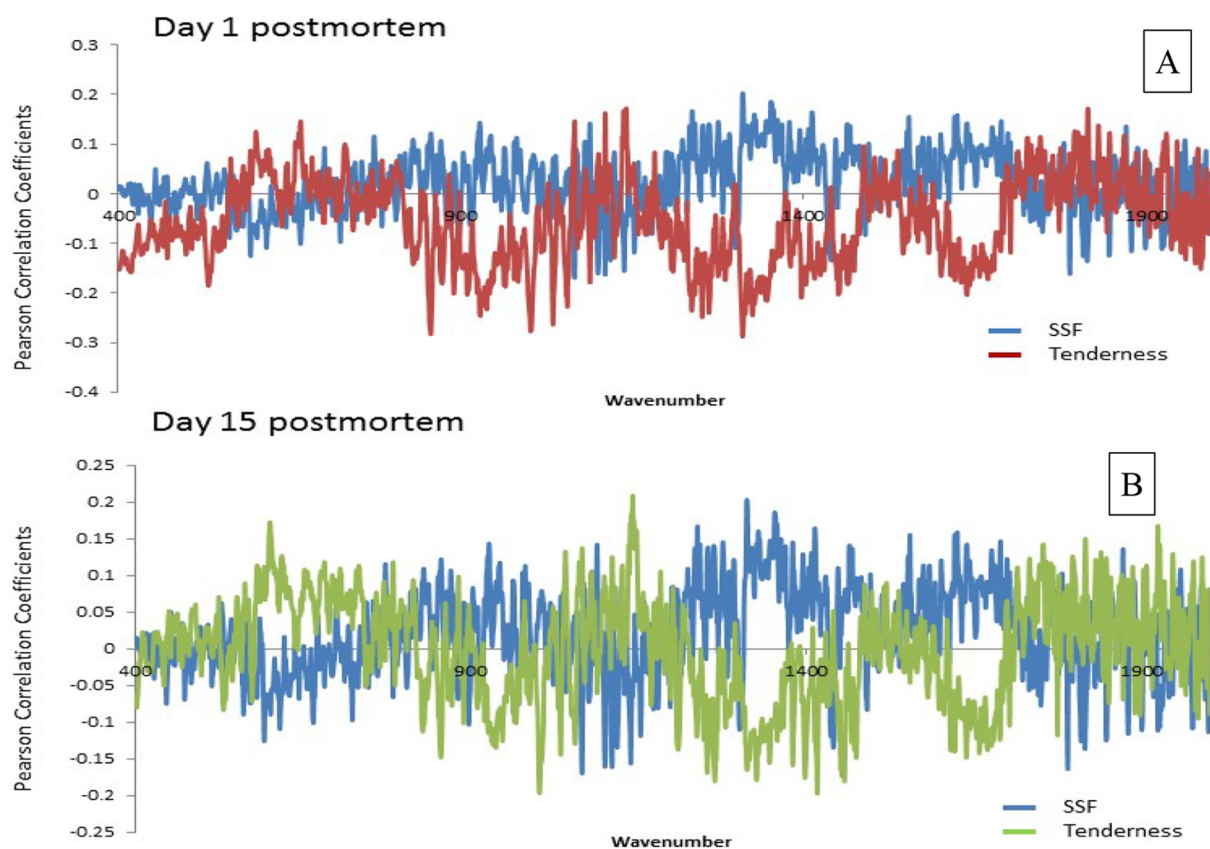


Fig. 4. Pearson Correlation Coefficients (r) between Raman (iRaman, B&W Tek, Newark, DE) spectral data and sensory tenderness and Slice Shear Force (SSF); n = 300 samples; a. d1 postmortem; b. d15 postmortem).

Table 1

Accuracy of the PLS regression prediction for sensory tenderness and SSF (Slice Shear Force) with different error tolerance, for samples at d 1 postmortem and 15 postmortem.

Error tolerance		± 25%	± 50%	± 100%
Tenderness	Day 1	50.1%	61.5%	92.3%
	Day 15	75%	98.1%	100%
SSF	Day 1	30.8%	57.5%	88.5%
	Day 15	88.5%	98.1%	100%

Table 2

The average accuracies for classifying pork Raman (iRaman, B&W Tek, Newark, DE) spectra into 4 groups based on percentiles. The average accuracies are calculated from 10 training and testing using Support Vector Machine.

Grouping	1st 25% percentile	2nd 25% percentile	3rd 25% percentile	4th 25% percentile
D1 postmortem tenderness	76.3%	62.4%	67.7%	68.6%
D15 postmortem tenderness	93.5%	90.1%	92.2%	95.5%
D1 postmortem SSF	76.1%	73.5%	72.6%	69.9%
D15 postmortem SSF	92.8%	93.1%	96.7%	100%

certainly not high enough for practical application. For day 15 post-mortem samples however, a substantial improvement in classification accuracies was observed. Consistent with what we have observed for the regression modeling results, spectral signatures of day 15 post-mortem samples correlated much better with either tenderness scores or SSF values. Aging is having a significant effect on the samples, the exact mechanism of which needs further investigation.

Table 3

The average classification accuracies for pork Raman (iRaman, B&W Tek, Newark, DE) spectra between Poor (1st 25% percentile for tenderness) and Good (4th 25% percentile for tenderness), for samples at day 1 and day 15 postmortem. The average accuracies are calculated from 10 training and testing using Support Vector Machine.

		Poor	Good
Classified as “poor”	Day 1	76.3%	5.1%
	Day 15	93.5%	1.5%
Classified as “good”	Day 1	4.8%	68.6%
	Day 15	2.3%	95.5%

Accuracies for crossing prediction into the incorrect extreme categories (good vs. poor) for tenderness ratings are shown in Table 3. Even for the day 1 postmortem samples, the SVM model performed well in not classifying the really tough meat (“poor”) into tender category (“good”), and vice versa. For day 15 postmortem, the classification of good and bad samples into their appropriate categories was reasonably good, and the cross prediction into these extreme categories was rare (< 2.5%).

We also investigated the effect of changing the definition of the grade categories on the classification accuracy. For the 800 samples that we had SSF values, we defined categories based on their SSF values, not on percentiles. As stated above, “good” was defined as SSF value < 10, “medium” 10–15, and “poor” above 15. The overall prediction accuracies for SSF value-based grouping was listed in Table 4. Apparently, pork samples that belong to the medium quality category are more difficult to predict based on their Raman spectroscopic characteristics. We also observed the same trend, that day 1 postmortem samples only provide moderately accurate classification results.

Another observation, which was consistent with our earlier report

**Table 4**

The average classification accuracies for pork Raman (iRaman, B&W Tek, Newark, DE) spectra ( $n = 800$ ) between Poor ( $SSF > 15$ ), Medium ( $10 < SSF < 15$ ) and Good ( $SSF < 10$ ). The average accuracies are calculated from 10 training and testing using Support Vector Machine.

Grouping	Good	Medium	Poor
Day 1	71.7%	67.2%	78.6%
Day 15	92.7%	85.8%	97.5%

(Wang et al., 2012), was that the prediction accuracy for “good” samples, either for tenderness or SSF, was consistently better than that for “poor” samples. It is hypothesized that the proteolysis of the muscle fibers results in more distinguishable Raman band structures in protein bands that may be highlighted in the barcode calculation. Further study is needed to identify these biochemical compositional markers that differentiate pork samples.

#### 4. Conclusions

In this report, Partial Least Squares Regression models were developed to predict the value of sensory tenderness scores and SSF values based on Raman spectroscopic characteristics of pork loins. It was demonstrated that sensory tenderness attributes of pork loins were lowly correlated to their Raman spectroscopic characteristics. Furthermore, a classification model was created to classify pork loins into grades by sensory tenderness and SSF values based on spectral data acquired with a portable Raman spectrometer rapidly enough to potentially be applied in an online situation. The method was demonstrated to yield moderate performance in identifying pork loins that belong to extreme categories of their sensory tenderness (i.e., superior and inferior) with freshly cut loins. The classification became much more accurate as the spectra from aged samples were used to match the time of the sensory evaluation and SSF measurements. The results of the report suggest that Raman spectroscopy, in combination with performance enhancing data processing and multivariate statistical discriminant modeling, has the potential to become a rapid on-line screening tool for pork quality.

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#### References

Beattie, R., Bell, S., Borggaard, C., & Moss, B. W. (2008). Preliminary investigation on the

- effects of aging and cooking on the Raman spectra of porcine longissimus dorsi. *Meat Science*, 80(4), 1205–1211.
- Beattie, R., Bell, S., Farmer, L., Moss, B., & Patterson, D. (2004). Preliminary investigation of the application of Raman spectroscopy to the prediction of the sensory quality of beef silverside. *Meat Science*, 66(4), 903–913.
- Bendall, J. R., & Swatland, H. J. (1988). A review of the relationship of pH with physical aspects of pork quality. *Meat Science*, 24(2), 85–126.
- ElMasry, G., Suna, D. W., & Allen, P. (2011). Non-destructive determination of water-holding capacity in fresh beef by using NIR hyperspectral imaging. *Food Research International*, 44, 2624–2633.
- Emerson, M. R., Woerner, D. R., Belk, K. E., & Tatum, J. D. (2013). Effectiveness of USDA instrument-based marbling measurements for categorizing beef carcasses according to differences in longissimus muscle sensory attributes. *Journal of Animal Science*, 91(2), 1024–1034.
- Fowler, S. M., Schmidt, H., Ven, R. V. D., Wynn, P., & Hopkins, D. L. (2014). Raman spectroscopy compared against traditional predictors of shear force in lamb m. Longissimus lumborum. *Meat Science*, 98(4), 652–656.
- Herrero, A. M. (2008a). Raman spectroscopy a promising technique for quality assessment of meat and fish: A review. *Food Chemistry*, 107(4), 1642–1651.
- Herrero, A. M. (2008b). Raman spectroscopy for monitoring protein structure in muscle food systems. *Critical Reviews in Food Science and Nutrition*, 48(6), 512–523.
- Honikel, K. O., & Fischer, C. (1977). A rapid method for the detection of PSE and DFD porcine muscles. *Journal of Food Science*, 42(6), 1633–1636.
- Hoving-Bolink, A. H., Vedder, H. W., Merks, J. W. M., de Klein, W. J. H., Reimert, H. G. M., & Frankhuizen, R. (2005). Perspective of NIRS measurements early post mortem for prediction of pork quality. *Meat Science*, 69(3), 417–423.
- Joo, S. T., Kauffman, R. G., Kim, B. C., & Kim, C. J. (1995). The relationship between color and water-holding capacity in post-rigor porcine longissimus muscle. *Journal of Muscle Foods*, 6(3), 200–226.
- Kamruzzaman, M., ElMasry, G., Sun, D. W., & Allen, P. (2012). Prediction of some quality attributes of lamb meat using near-infrared hyperspectral imaging and multivariate analysis. *Analytica Chimica Acta*, 714, 57–67.
- Kauffman R.G., & Warner, R.D. (1993). Evaluating pork carcasses for composition and quality. G.R. Hollis, Growth of the pigs (p.141–166). Wallingford, UK: CAB International.
- Kemsley, E. K. (1998). *Discriminant analysis and class modeling of spectroscopic data*. Chichester, UK: John Wiley & Sons 13–16.
- King, D. A., Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2009). Comparison of palatability characteristics of beef gluteus medius and triceps brachii muscles. *Journal of Animal Science*, 87, 275–284.
- Martens, H., & Naes, T. (1992). *Multivariate calibration*. John Wiley & Sons Inc.
- Olsen, E. F., Rukke, E. O., Flåtten, A., & Isaksson, T. (2007). Quantitative determination of saturated, monounsaturated and polyunsaturated fatty acids in pork adipose tissue with non-destructive Raman spectroscopy. *Meat Science*, 76(4), 628–634.
- Schmidt, H., Scheier, R., & Hopkins, D. (2013). Preliminary investigation on the relationship of Raman spectra of sheep meat with shear force and cooking loss. *Meat Science*, 93(1), 138–143.
- Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (1999). Evaluation of slice shear force as an objective method of assessing beef longissimus tenderness. *Journal of Animal Science*, 77, 2693–2699.
- Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2004). Technical note: Use of belt grill cookery and slice shear force for assessment of pork longissimus tenderness. *Journal of Animal Science*, 82, 238–241.
- Steinwart, I., & Christmann, A. (2008). *Support vector machines*. New York: Springer Verlag.
- Toldrá, F., & Flores, M. (2000). The use of muscle enzymes as predictors of pork meat quality. *Food Chemistry*, 69(4), 387–395.
- van Laack, R. L., Kauffman, R. G., Sybesma, W., Smulders, F. J., Eikelenboom, G., & Pinheiro, J. C. (1994). Is color brightness (L-value) a reliable indicator of water-holding capacity in porcine muscle. *Meat Science*, 38(2), 193–201.
- Wang, Q., Lonergan, S. M., & Yu, C. (2012). Rapid determination of pork sensory quality using Raman spectroscopy. *Meat Science*, 91(3), 232–239.
- Warner, R. D., Kauffman, R. G., & Greaser, M. L. (1997). Muscle protein changes post mortem in relation to pork quality traits. *Meat Science*, 45(3), 339–352.