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PREDICTION OF THE TENDERNESS OF COOKED POULTRY PECTORALIS MAJOR MUSCLES BY NEAR-INFRARED REFLECTANCE ANALYSIS OF RAW MEAT.

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Abstract:

Tenderness in boneless poultry breast meat is of utmost importance to consumers. However, there is currently no non-destructive method to predict poultry breast meat tenderness. Textural properties of poultry breast meat were predicted by near-infrared reflectance (NIR) spectroscopy. Spectra were collected on 390 poultry Pectoralis major muscles from broiler carcasses to examine the correlation between NIR spectroscopy and the Meullenet-Owens Razor Shear (MORS) test. Two instrumental parameters (maximum shear force and total shear energy) were used as estimates of meat tenderness. Calibration (R^2_{cal}) and validation (r^2_{val}) coefficients of determination were used for predicting instrumental measurements using the reflectance, and its first and second derivatives. The model using second derivative reflectance data yielded the best results for all samples. Regressions performed on these samples produced R^2_{cal} values ranging from 0.70 to 0.75 and r^2_{val} values ranging from 0.59 to 0.65 for maximum shear force and total shear energy, demonstrating the potential of NIR to predict poultry breast meat tenderness. These findings could have a practical importance as this method could qualify NIR for an on-line assessment to sort poultry breast meat according to tenderness levels.

Introduction:

Many techniques exist for assessing meat tenderness in poultry and other meats. Either instrumental (e.g. Allo-Kramer method and Warner-Bratzler shears) or sensory evaluation tests can provide reliable information (Bouton and Harris, 1972; Huff and Parrish, 1993; Lyon and Lyon, 1990, 1991, 1997; Shackelford et al., 1991; Shackelford, Wheeler and Koohmaraie, 1997; Smith et al., 1988; Yacowitz, Davis, and Jones, 1978) on meat tenderness. However, these techniques are destructive, time consuming, and unsuitable for on-line applications. Developing fast, non-destructive, accurate, and on-line techniques are greatly desired in modern day processing plants. Near-infrared spectroscopy (NIR) can possibly step into this role due to the speed and ease of use.

Near-infrared reflectance is an analytical technique that has been used over the years for quality management of beef, pork, and chicken (Lyon, 2002). Applications have not only included prediction of chemical, textural, and sensory characteristics of meats, but attempts have been made to classify beef according to tenderness levels (Park et al., 1998; Rodbotten et al., 2001). However, the results of these studies were inconsistent at best. Now, using a non-destructive, fiber optic probe, there is a unique opportunity for developing an on-line assessment with the potential of sorting poultry meat by tenderness levels.

The objective of this study was to evaluate the use of NIR to predict poultry *Pectoralis major* muscle texture by assessing the relationship between NIR spectral data and an instrumental razor blade shear test, an instrumental measurement previously found to have a high correlation with the human perception of meat tenderness.

Materials and methods:

Meat samples

The birds were obtained from poultry nutrition trials conducted by the University of Arkansas Poultry Research Farm. A total of 195 commercial age broilers were slaughtered at seven weeks of age, and all birds were housed under commercial growing conditions until time of slaughter. Birds were hung on a shackle line and processed commercially in order to obtain the varying range of tenderness in the breast fillets commonly seen in processing applications. Birds were electrically stunned (11 V, 11 mA, 10 s), manually cut (severed left carotid artery and jugular vein), bled out (1.5 min), scalded (55 °C, 2 min) and picked in-line using commercial defeathering equipment.

Following processing, broiler carcasses were chilled using a two-stage chilling system consisting of a 0.25 h pre-chill at 13 °C, followed by an agitated ice-slush chill at 1 °C for 0.75 h. At the time of deboning, both right and left *Pectoralis major* muscles were excised by severing the humeral-scapular joint,

and pulling downward on the wings according to Hamm (1981). Post-mortem deboning time has been shown to be a process parameter influencing the tenderness of *Pectoralis major* muscles (Lyon and Lyon, 1990), therefore it was selected as a process variable. Post-mortem deboning times for the samples (n=390) were 0.25 h (hot-boned), 1.25 h, 2 h, 2.5 h, 3 h, 4 h, 6 h, and 24.0 h post-mortem (PM). The deboning treatments were applied to gain a definite and varying level in meat tenderness. Carcasses for 1.25 h to 24 h PM were aged on ice after the chilling period until deboning. The 0.25 h PM samples were not aged on ice as they were deboned hot.

After aging, the *Pectoralis major* muscles from all carcasses within the deboning treatment were individually placed into coded plastic bags. The bags were then put on ice, and taken directly from the processing plant to the site of the NIR analysis. This was done so as to simulate normal processing practices before NIR assessment of meat tenderness. It was feared that freezing the samples would hinder the potential of NIR spectroscopy and while the results have not been compared (fresh vs. thawed) the samples were not frozen because it is not a common processing practice.

NIR spectroscopy

Raw samples were scanned shortly after deboning using a fiber optic probe (NIRSystems 6500) equipped with the software ISIScan. Each breast was placed on a flat surface and scanned twice at the top of the muscle by holding the probe perpendicular to the breast meat. These two measurements were then averaged to obtain the spectra data for the chicken samples. Samples were scanned in reflectance mode from 400 to 2498 nm at 2nm increments. This was done for both breast samples for each bird.

Instrumental texture analysis

After NIR analysis, the breast meat was refrigerated at 4°C for approximately 60 h. The meat was held at refrigeration to again simulate common practices by poultry processors. After the period had elapsed, the samples were cooked in coded aluminum lined and covered pans at 176.7°C for 36 minutes or until an internal temperature of 76°C was reached. The internal temperature was measured using a digital thermometer (Model HT1000, Cooper Instruments Industrial Thermometers and Temperature Instrumentation, Ontario, Canada).

Each sample was analyzed for textural characteristics with a TA.XT2Plus texture analyzer (Texture Technologies, Scarsdale, NY, USA). A razor blade shear test (MORS) recently developed in our laboratories and described by Cavitt et al. (2001, 2004) was used. This test consists of shearing the sample perpendicularly to the longitudinal fiber orientation with a 0.5 mm thick, 8.9 mm wide, and 30 mm long straight edge razor blade (#17, Excel, Paterson, NJ, USA). Experiments were conducted to determine

the extent of the blade's sharpness. This was done by monitoring the force necessary to cut a piece of piece of 20lb paper as a function of the number of shear tests performed on *Pectoralis major* muscles. It was determined that a blade could be used for up to 100 shears without seeing an increase greater than 5% in shear force. Conservatively, the blade was replaced after every 50 shears. After proper calibration of the texture analyzer, the cooked sample was manually placed on a flat aluminum plate featuring a 1x50 mm slot (used to prevent the blade from coming into contact with the aluminum plate) and a total of four measurements were made on each of the cooked samples. The crosshead speed was set at 10 mm/s. The sample shear depth was set at 20 mm which had been previously determined as an optimal depth for fully shearing a sample. In addition, to ensure accurate and consistent measurements, each test was started with a contact force of 0.1 N. The instrumental data was collected using Texture Exponent 32 version 1.0.0.92 (Stable Micro Systems, Godalming, Surrey, UK). For each shear, a force-deformation curve (force vs. time) was obtained. Maximum shear force (F, N), and total shear energy (E, N.mm) were calculated using the macro options of Texture Exponent.

Data analysis

NIR and texture analyzer data were processed using the multivariate regression software Unscrambler (Version 8.1, Camo, AS, Norway). The mean values for the texture and reflectance data, as well as the spectra's first and second derivative spectrum data were used to develop predictive models using the Partial Least Squares (PLS-I) and Jack-knife optimization options of Unscrambler. The first and second derivative was obtained using the Savitzky Golay algorithm. Jack-knifing is a procedure designed to test the significance of the model and is preformed during full-cross validation. Full-cross validation was used to assess the robustness and the discrimination ability of the predictive models. RMSEC (root mean square error of calibration) and RMSEP (root mean square error of prediction) were used to compare the goodness-of-fit of the models. RMSEP is the average difference between predicted and observed response values at the prediction stage, while RMSEC shows differences at the calibration stage. Additionally, the ratio (RMSEP/RMSEC) was used as an indicator of model robustness. The model is said to be robust if values are found to be close to 1.0, but less than 1.2. Also, the relationship between the standard deviation of instrumental parameters (S_{tot}) and the RMSEP values were assessed to determine the discrimination ability of the model. Large values (>2) indicate adequate discrimination.

Results:

The instrumental texture data indicates that that tenderness improved with increasing deboning time, a similar result to that reported by Cavitt et al. (2004). Values for total energy (TE)

decreased from a maximum at the 0.25hr deboning time of 194.9N.mm to a minimum of 127.3N.mm for the 24hr deboning time. A sharp decrease in TE was observed after 2.5hrs from 190.8 N.mm to 154N.mm at the 4hr deboning time.

For the first derivative of the reflectance spectra, the model statistics were less satisfactory. Calibration and validation coefficients ($R_{cal} = 0.59$ and 0.68 $R_{val} = 0.54$ and 0.63) were found to be less than those models using the second derivative spectra. However, as seen in Table 1, the use of the reflectance spectrum as a predictor of instrumental texture measurements yielded slightly better calibration coefficients ($R_{cal} = 0.62$ and 0.69) than the first derivative spectra curve. Appropriately, validation correlation coefficients were also slightly better ($R_{val} = 0.55$ and 0.64) than the first derivative spectra.

The best modeling results were obtained when using the second derivative of the reflectance data. For all samples (all deboning times), the modeling yielded modest results (see Table 1).

	Total Energy to Max Force			Total Shear Energy (Nmm)		
	R ²	R(1st) ¹⁰	R(2nd) ¹¹	R	R(1st)	R(2nd)
n ¹	396					
PC ²	12	4	4	12	4	4
R ² ³	0.62	0.59	0.70	0.69	0.68	0.75
r ² _{val} ⁴	0.55	0.54	0.59	0.64	0.63	0.65
RMSEP ⁵	26.79	25.95	25.10	27.35	26.77	26.35
RMSEC ⁶	24.79	24.66	21.81	25.08	25.35	22.75
Robustness ⁷	1.08	1.05	1.15	1.09	1.05	1.15
DA ⁸	1.24	1.25	1.41	1.37	1.36	1.51
1 - Number of samples				7 - Model robustness = RMSEP/RMSEC		
2 - Optimal Number of Principal Components				8 - Discrimination Ability DA = SD/RMSEP		
3 - Calibration Coefficient of Determination				9 - R = Reflectance Spectra (400-2498nm)		
4 - Validation Coefficient of Determination (full cross-validation)				10 - R(1st) = first derivative of R (1/nm)		
5 - Root Mean Square Error of Prediction (RMSEP) (full cross-validation)				11 - R(2nd) = second derivative of R (1/nm ²)		
6 - Root Mean Square Error of Calibration (RMSEC)						

Table 1. Model statistics for the prediction of cooked poultry breast meat texture from NIR spectroscopy

	Observed Tender	Observed Tough
Total	239	150
Predicted Tender	229	55
Predicted Tough	10	95
% Correct	95.8	63.3

Table 2. Classification of Pectoralis major muscles by NIR spectroscopy according to tenderness categories.

Calibration and validation coefficients (R_{cal} and R_{val}) were found to be acceptable ($R_{cal} = 0.70-0.75$ $R_{val} = 0.59-0.65$) for total energy (TE) and total energy to max force (TE to MF), and RMSEP as well as RMSEC values were relatively low. Robustness for TE and TE to MF were 1.150 and 1.158, respectively. Thus, the models were said to be robust. Accordingly, the discrimination ability (DA) of the model was found to be slightly low 1.41 (TE to MF) and 1.51 (TE), and the model could not be deemed highly discriminative. This

demonstrates that some model improvements are needed in order to develop a more discriminative model. As seen above, TE and TE to MF, both measured in Nmm, were found to be well predicted. However, max force was found to be unsatisfactorily predicted by NIR spectroscopy. Fortunately, previous research (Cavitt et al., 2004) found TE to be the best predictor of poultry breast meat tenderness and max force to be the least accurate in predicting tenderness.

Weighted regression coefficients helped in identifying the important from the unimportant spectral regions for predicting tenderness by examining the relationship between variables X and Y. Small absolute values (close to 0) are indicative of an unimportant variable, while larger absolute values indicate a variable of large importance. This coefficient value is the average increase in Y when the corresponding X variables are increased by one unit, holding all other variables constant (Camo, 1999). Upon examination of these regression coefficients it was concluded that the spectral regions of 400-600 nm, 950-1,212 nm, and 1,770-1,950 nm were important regions for predicting tenderness in poultry breast meat (See Figure 1). The spectral region of 400-600 nm has been shown in the literature to be highly correlated to myoglobin, whereas the region ranging 1,770 nm to 1,950 nm are closely related to water, proteins, and fats, respectively. These two spectral regions, as seen in figure 1, are positively correlated for tenderness. However, the region from 950 nm to 1,212 nm was shown to be negatively correlated with tenderness. From this model data, average spectra curves for the various debone times were calculated and an average prediction of instrumental force was found and compared to that of the actual averages (See Figure 2). This model yielded excellent results with a R² value of 0.97. Razor blade force values were closely, and in some cases exactly, predicted using this model and it seems NIR can be used to accurately predict average razor blade shear force for a deboning time.

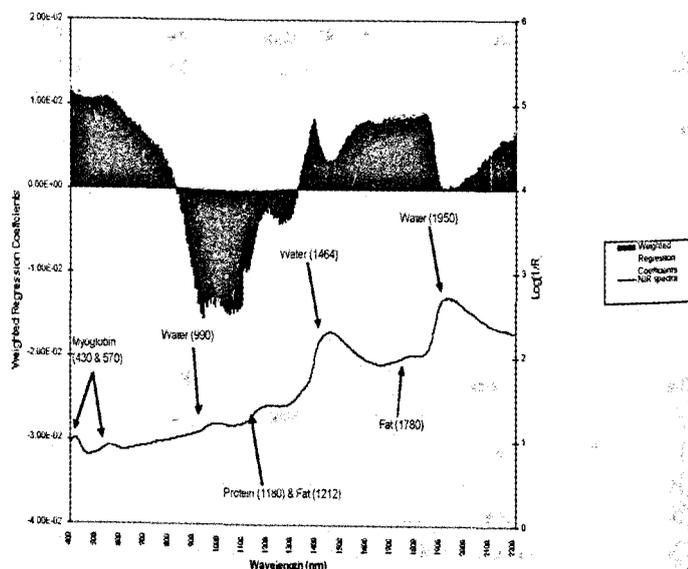


Figure 1. Weighted regression coefficients and corresponding spectra for the model predicting Total Energy from the 2nd derivative of the NIR spectra.

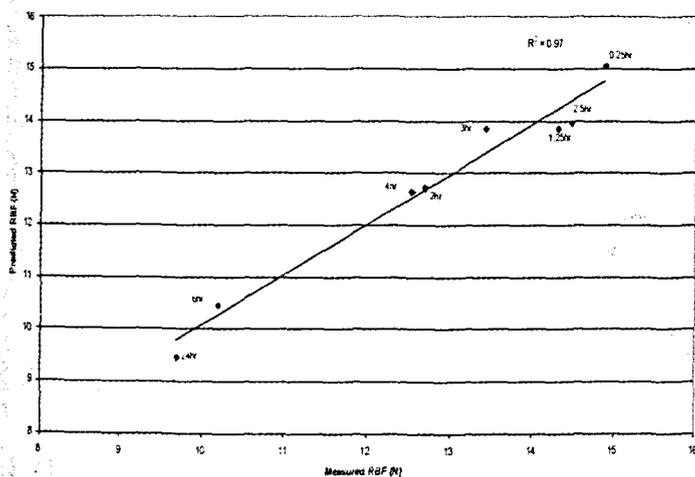


Figure 2. Measured and predicted values from the model for various deboning times.

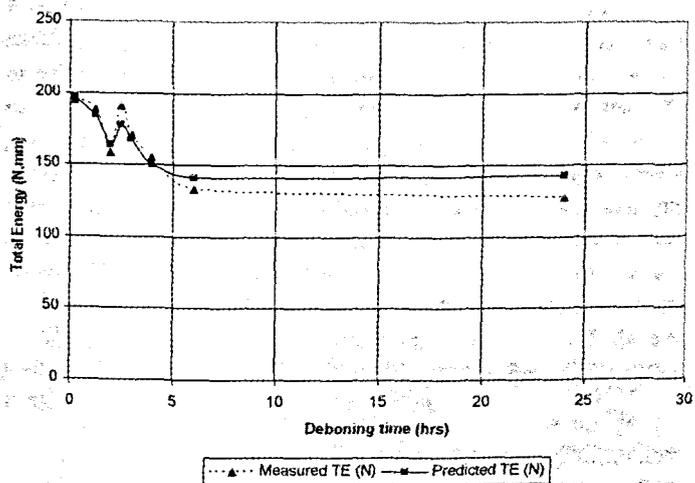


Figure 3. Mean scores for total energy and predicted values from the model using NIR data at various deboning times.

Discussion:

The results obtained demonstrate that NIR spectroscopy has great potential for predicting poultry breast meat tenderness. These encouraging results were comparable to prior research conducted by Park et al. (1998) for the prediction of beef muscle tenderness using NIR data. Park, using similar regression techniques, yielded an R^2_{cal} of 0.82. In this present study, results were slightly less, but still satisfactory with calibration R^2 of 0.70 and 0.75 for TE to MF and TE, respectively.

The second derivative of the reflectance data is recommended for optimizing the correlation between NIR and instrumental measurements for breast meat. The smallest RMSEP's and largest discrimination indices were reported along with the second derivative spectra curve. The mean values for TE at the different deboning times were compared to the mean predicted values using NIR spectroscopy (See Figure 3). Upon examination of these numbers it was concluded that NIR was

very successful in predicting the tenderness of samples at lower deboning times (theoretically, the more tough samples). As seen in figure 3, the 0.25 h and 1.25 h samples predicted means were very close (< 4 Nmm) to the actual means for TE. However, as deboning time increased NIR spectral data had a more difficult time predicting TE. For the 6 h and 24 h samples, actual versus predicted mean score ranges were more varied (> 8 Nmm) than those of the shorter debone times. Thus, NIR spectroscopy is more suitable for the prediction of tougher (3 h PM debone or less) meat than it is for more tender (> 3 h debone) meat.

From the data presented, it seems that NIR could be utilized for on-line assessment of meat tenderness, and certainly the models are acceptable for the possible classification of muscles according to tenderness levels. To verify that statement, muscles were classified into two categories (tender and tough). Values were predicted by cross-validation from NIR data, and the muscles were then classified as tender or tough according to the same value of TE. The value of 177 Nmm was chosen as the cut-off between tender and tough filets based on previous findings in our labs. The percentages of correctly classified samples were then calculated for both the tender and tough categories (see table 2). Results show that out of 239 tender samples NIR was able to predict 95.8%, or 229 samples, accurately. Accordingly, out of 150 tough samples NIR predicted 95, or 63.3%, correctly. It seems that this data, especially for the prediction of tenderness, is acceptable. For instance, before classification only 61.4% of breast filets would have been classified as "tender", while 38.5% would have been classified as "tough". However, after NIR classification, a total of 284 out of 389 filets or 80.6% would be classified as tender, while only 19.4% would qualify as tough. An increase of 20% of tender filets after NIR analysis would certainly justify its use in large poultry processing facilities where marination applications are widely used to achieve a more tender product. Additionally, facilities that run large quantities of breast meat could see efficiency increase and cost decrease as marination may not be needed for as much as 20% of their product.

Further research should concentrate on estimating correlations between sensory measures of tenderness and NIR spectroscopy to confirm the potential of this non-destructive method.

Conclusion:

These results demonstrate that NIR spectroscopy has the ability to predict poultry breast meat tenderness; this would qualify NIR as a screening method for sorting breast meat according to tenderness levels. However, more testing must be done to develop robust predictive models and to test the consistency of NIR analysis. In addition, new NIR equipment (more suitable for on-line applications) could be tested as many new NIR spectroscopy applications are now becoming available.

Eventually, on-line NIR non-destructive applications should be tested in a small processing facility to evaluate NIR's performance and durability for large scale processing plant applications.

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