Prediction of meat quality traits in Nelore cattle by near-infrared reflectance spectroscopy¹

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ABSTRACT: The main definition for meat quality should include factors that affect consumer appreciation of the product. Physical laboratory analyses are necessary to identify factors that affect meat quality and specific equipment is used for this purpose, which is expensive and destructive, and the analyses are usually time consuming. An alternative method to performing several beef analyses is near-infrared reflectance spectroscopy (NIRS), which permits to reduce costs and to obtain faster, simpler, and nondestructive measurements. The objective of this study was to evaluate the feasibility of NIRS to predict shear force [Warner-Bratzler shear force (WBSF)], marbling, and color (*a = redness; b* = yellowness; and L* = lightness) in meat samples of uncastrated male Nelore cattle, that were approximately 2-yr-old. Samples of *longissimus thoracis* (n = 644) were collected and spectra were obtained prior to meat quality analysis. Multivariate

calibration was performed by partial least squares regression. Several preprocessing techniques were evaluated alone and in combination: raw data, reduction of spectral range, multiplicative scatter correction, and 1st derivative. Accuracies of the calibration models were evaluated using the root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP), coefficient of determination in the calibration (R²C), and prediction (R²P) groups. Among the different preprocessing techniques, the reduction of spectral range provided the best prediction accuracy for all traits. The NIRS showed a better performance to predict WBSF (RMSEP = 1.42 kg, R²P = 0.40) and b* color (RMSEP = 1.21, R²P = 0.44), while its ability to accurately predict L* (RMSEP = 1.98, R²P = 0.16) and a* (RMSEP = 1.42, R²P = 0.17) was limited. NIRS was unsuitable to predict subjective meat quality traits such as marbling in Nelore cattle.

Key words: marbling, meat color, preprocessing techniques, shear force

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INTRODUCTION

Several definitions of meat quality exist but, according to Williams (2008), the main definition should include factors that affect consumer appreciation of the product. Among these factors, meat tenderness is considered a determinant trait for consumer satisfaction since consumers are willing to pay more for a better-quality product, i.e., they would pay a premium for a steak with guaranteed tenderness (Shackelford et al., 2001). Fat content is also important because it affects flavor, juiciness, and possibly toughness (Williams, 2008). Finally, meat color is an essential visual attribute that consumers consider for purchasing (Liu et al., 2003). Physical laboratory analyses are necessary to identify factors that affect meat quality. Specific equipments are used for this purpose, which are expensive and destructive, and the analyses are usually time consuming (Leroy et al., 2003). For example, tenderness, analyzed by Warner-Bratzler shear force (WBSF) requires about 24 h, while fat can be analyzed by marbling score, usually faster than WBSF, or by chemical analysis, which determine true extractable lipid content, but much greater time is required. Meat color is faster than WBSF and fat content, but specific equipment is necessary to perform this analysis.

An alternative method to performing several beef analyses is the near-infrared spectroscopy (NIRS), which permits to reduce costs and to obtain faster, simpler, and nondestructive measurements (Liu et al., 2003; Prevolnik et al., 2004). For this technique, a spectrometer of reflectance in the near-infrared region is used, which consists of an optical reading chamber that produces the spectra and software to develop the statistical models (Campestrini, 2005). Calibration equations are estimated and validated by regression analysis and, if the equation is accurate (prediction ability of the equation), it is possible to predict new values for meat quality without the need for standard laboratory analysis.

The objective of the present study was to evaluate the feasibility of NIRS to predict tenderness, marbling, and meat color in Nelore beef.

MATERIAL AND METHODS

Meat Samples Analysis

A total of 644 meat samples were obtained from approximately 2-yr-old uncastrated male Nelore cattle at 3 farms. The animals were raised on pasture and feedlot finished for approximately 90 d prior to

slaughter in a commercial slaughterhouse, under the approval of ethics committee of the São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences, Jaboticabal – SP, Brazil (N° 18.340/16). After slaughter, the carcasses were cooled in a cold storage chamber (0 to 2 °C) for 48 h postmortem. Samples of *longissimus thoracis* muscle (2.54 cm thick) with bone were taken between the 12th and 13th rib of the left half-carcasses and vacuum packaged, frozen at –20 °C.

To perform the analyses, the process of samples thawing was performed: samples were transferred from freezer to a refrigerator for 12 h; the samples were removed from the vacuum package and kept at room temperature (~25 °C). The meat temperature was monitored and when the temperature reached 5 °C the NIR spectra were obtained using a Spectrum 100N FT-IR spectrometer (PerkinElmer, Shelton, USA) equipped with a fiber optic probe accessory. Spectral data were collected as relative absorbance in a wavenumber range from 4,000 to 10,000 cm⁻¹ (2,500 to 1,000 nm) range at 2 cm⁻¹ intervals and 64 scans. The mean spectrum of each sample was obtained averaging 6 spectra per sample and the absorbance data were stored as log 1/R (R = reflectance). Spectra collection and reference analysis (meat quality analysis) were performed on the same sample.

Meat quality was analyzed immediately after collection of the NIR spectra. A visual grading system (USDA, 1989) was used to determine the marbling score of the *longissimus thoracis* muscle samples, which received scores ranging from 0 to 9, where 1 = practically absent; 2 = traces; 3 = slight; 4 = small; 5 = modest; 6 = moderate; 7 = slightly abundant; 8 = moderately abundant; 9 = abundant. Meat color (L*, lightness; a*, redness; b*, yellowness) was measured using CIELab system with a help of a Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Tokyo, Japan) with standard D65 light source, observer angle 10°, and aperture size of 5 cm, as described by Borges et al. (2014). Prior the evaluation, the equipment was calibrated using a black and a white standard plate and color readings were taken at 3 locations of the *longissimus thoracis* muscle sample. The average was then calculated from these measurements.

Finally, WBSF was performed using standard procedure proposed by Wheeler et al. (1995) in which the samples were baked until they reach an internal temperature of 71°C. The temperature was measured using thermocouple wires (Equipe, Sorocaba-SP, Brazil) inserted into the center of each steak and coupled to the temperature recorder. Shear force was measured with a Warner-Bratzler Shear

Force machine (GR-Manufacturing, Kansas, USA). Shearing was performed on 1.27 cm in diameter removed longitudinally to the muscle fibers. Eight measurements were collected per sample to increase precision of the results, expressed as kilogram (kg).

Chemometrics

The calibration set was composed by 644 NIR spectra obtained from meat samples of different Nelore animals (independent matrix **X**). The dependent matrix (**Y**) was composed by the information coming from the analyzed meat quality traits (reference analysis) WBSF, marbling, and color (a^* , b^* , and L^*). Multivariate calibration was performed using partial least squares regression (PLSR) with full cross-validation using the software Unscrambler X.1 (CAMO Software, Oslo, Norway).

To improve the accuracy, several preprocessing techniques were tested. The techniques using raw spectra and spectral preprocessing techniques were evaluated alone and in combination. The spectral preprocessing techniques applied to the spectra were performed according to (Nicolaï et al., 2007) and consisted of raw data (**RAW**), reduction of spectral range (**RSR**), normalization by multiplicative scatter correction (**MSC**), and 1st derivative (1D) transformation. In **RAW**, all collected spectra were used and only “mean centering” was considered, which is a standard procedure used to subtract the mean from each variable, thus ensuring that all results are interpreted in terms of variation around the mean. In **RSR**, only wavenumbers ranging from 2,420 to 1,097 nm were selected to reduce the spectral noise that is found at the beginning and at the end of the original spectra (4,000 to 10,000 cm^{-1}). For normalization, **MSC** was used which removes the effects of light scattering by linearizing the spectrum. 1D transformation was performed using the Savitzky-Golay smoothing algorithm (Næs et al., 2004). This transformation was used to remove baseline shifts and superposed peaks.

The performance of the calibration models was evaluated by calculating the root mean square error of calibration (**RMSEC**) and root mean square error of prediction (**RMSEP**) using the following equation:

$$\text{RMSEC or RMSEP} = \sqrt{\sum_{i=1}^n \frac{(y_i - \hat{y}_i)^2}{n}} \quad (1)$$

where y_i is the reference value of sample i ; $\hat{y}_i$ is the value predicted by the model, and n is the number of samples. In addition, the coefficient of determination (R^2), which represents the proportion of

variance explained by the response variable in the calibration group (R^2C) and in the prediction group (R^2P), was calculated (Nicolaï et al., 2007).

RESULTS AND DISCUSSION

Meat Quality Evaluation

Among the studied traits, WBSF showed the highest coefficient of variation (30%) and the mean value was slightly above the limit of “tender” beef (Shackelford et al., 1997; Destefanis et al., 2008; Rodas-González et al., 2009; Table 1). Our WBSF results were slightly higher than the values reported in the literature for Nelore cattle meat (Bonin et al., 2014; Magalhães et al., 2016; Baldassini et al., 2017) and for *Bos taurus* beef (O'Connor et al., 1997; Riley et al., 2005), while marbling had a low coefficient of variation and the mean value was similar to previous determinations in Nelore beef (Francisco et al., 2015; Baldassini et al., 2017), which used the same marbling score as our study. Comparison marbling scores is difficult because of the use of different scoring systems. In the United States, 11 marbling scores are considered and each score resulted in over a 100-point scale (Burrow, 2001), or 9 marbling scores are also used, as we used in this study (USDA, 1989; USDA, 2016). While in Australia, the score ranges from 1 to 7. In these systems, the meat samples are obtained between the 12th and 13th rib. The Japanese system uses 12 marbling scores collected between the 6th and 7th rib (Burrow, 2001).

Regarding meat color traits, the coefficients of variation were intermediate for a^* and b^* and low for L^* . The L^* parameter indicates lightness, where 0 corresponds to black color and 100 to white color. The mean value found in this study was similar to that reported for Nelore cattle (Costa et al., 2013; Baldassini et al., 2017) and *Bos Taurus* (Cecchinato et al., 2011; Prieto et al., 2014), but lower than that

Table 1. Descriptive statistics for meat quality traits ($n = 644$) in Nellore beef

Trait ¹	Mean	Standard deviation	Coefficient of variation (%)
WBSF (kg)	6.15	1.84	30
Marbling	2.96	0.15	5
a^*	9.37	1.56	17
b^*	10.49	1.62	15
L^*	34.93	2.16	6

¹WBSF = Warner-Bratzler shear force; a^* = redness; b^* = yellowness; L^* = lightness.

obtained by Baldin et al. (2013) and Bonin et al. (2014) also for the Nelore breed. For a^* parameter, a lower value corresponds to less red meat and a greater value to more red meat. Thus, the mean value found in this study corresponds to less red meat than those reported in the literature for the Nelore breed (Baldin et al., 2013; Costa et al., 2013; Baldassini et al., 2017). For b^* parameter, a lower value indicates less yellow meat and a higher value more yellow meat. The mean value found in this study was higher than that reported by Baldassini et al. (2017) and lower than Baldin et al. (2013) reported for Nelore breed. These differences in meat color can be attributed to the strong influence of the environmental factor in these traits, as *ante-mortem* and *postmortem* handling, i.e., chilling rate, packaging methods, time and temperature at storage, exposure to oxygen and microbial load, animal age, nutritional diet, glycogen storage, antioxidant accumulation, pH, oxygen consumption, and met-myoglobin activity (Faustman and Cassens, 1990; Mancini and Hunt, 2005).

NIR Spectra and Chemometric Analysis

Chemometric analysis was performed using raw data and the following preprocessing techniques: RSR, MSC, and 1D. Figure 1 depicts these spectral preprocessing techniques and Table 2 shows the results of PLSR for each preprocessing technique evaluated.

The NIR spectra of the meat samples exhibited the most intense absorption bands at 5,200 and 7,000 cm^{-1} , corresponding to the combination of O-H vibrations and first overtone of O-H stretching, respectively (Fig. 1). This trend is due to the presence of H_2O in the meat samples (Schwanninger et al., 2011) as the water content of beef is approximately 70.62% (Strasburg et al., 2008). Small absorption bands were present at 5,700 and 8,500 cm^{-1} , corresponding to the first and second overtone of C-H stretching, respectively (Fig. 1) (Schwanninger et al., 2011). Several factors can affect the spectral profile, masking its chemical information, such as light scattering, instrumental noise, tissue heterogeneities, ambient effects, and other sources of variability (Nicolai et al., 2007; Rinnan et al., 2009). Such effects can be attenuated by using preprocessing techniques, which improve the signal-to-noise ratio of spectral data (Hibbert, 2016). Additionally, the use of multivariate calibration techniques during chemometric analysis removes redundant information that does not contribute to sample variability in the original

data by reducing it to a few sets of variables that explain most of the variance (Hibbert, 2016). This approach maintains only the most important information during the calibration procedure.

Although Nicolai et al. (2007) reported that the NIR region covers the wavelength range from 780 to 2,500 nm, we used the range of 1,100 to 2,500 nm as done in other studies evaluating meat quality (Rødbotten et al., 2000; Prieto et al., 2008; Cecchinato et al., 2011). Studies have used different wavelength ranges to analyze meat quality, including the visible range (380 nm to 780 nm) (Leroy et al., 2003; Liu et al., 2003; Prieto et al., 2014), and the NIR range of 833 to 2,500 nm to evaluate meat quality in 2 modes: reflectance and transmission (Leroy et al., 2003). The authors concluded that the use of a wider wavelength range in NIRS (reflectance or transmission) did not improve the accuracy of predicting WBSF. For meat color (L^* and b^*), NIRS showed good potential in the reflectance mode.

Among the different spectral preprocessing techniques, the model considering RSR (Fig. 1c) provided the best predictions for all traits (Table 2), followed by RAW (Fig. 1a) and MSC (Fig. 1b). The worst results were obtained for the model using 1D without RSR (Fig. 1e). It should be noted that RSR provided good results when applied alone or in combination with other techniques. RSR in the range of 4,132 to 9,112 cm^{-1} was used to exclude extreme wavelengths from the spectral range, avoiding high noise levels and water absorbance which do not contribute to the accuracy of the prediction.

The reduction in data variability caused by MSC and 1D preprocessing (Fig. 1) resulted in low accuracy of the calibration (Table 2) for all traits studied. These preprocessing techniques may have been too rigorous for the data type used here and may have removed valuable information, reducing the accuracy of the calibration model.

Different results have been reported in other NIRS studies of meat quality traits, which found the MSC technique with first and second derivative and SNV-D (Standard Normal Variate and Detrend) (Prieto et al., 2008) to be more accurate for a^* and b^* than RAW and RSR. However, Prieto et al. (2014) obtained similar results for L^* considering only raw data (400 to 2498 nm). For WBSF, Prieto et al. (2008) identified MSC as the best preprocessing technique, while Prieto et al. (2014) reported SNV-D to be the best method. It is important to note that these studies were performed using different breeds, the reference traits were measured in different ways, and NIR spectra were obtained

Figure 1. NIR spectra (absorbance $\times$ wavenumber (cm^{-1})) obtained for the different preprocessing techniques: RAW = raw data (a); RSR = reduction of spectral range (b); MSC = multiplicative scatter correction (c); MSC + RSR (d); 1D = 1st order derivative (e); 1D + RSR (f); MSC + 1D (g); MSC + 1D + RSR (h).

in different wavelength ranges. Thus, the use of diverse data sets results in different accuracies of the prediction model, reinforcing the need to study the applicability of NIRS in the Nelore breed.

Using full cross-validation, the best WBSF calibration and prediction was obtained using

an NIR spectral window of 4,132 to 9,112 cm^{-1} (2,420 to 1,097 nm) since this window provided lower calibration (RMSEC = 1.34 kg) and prediction errors (RMSEP = 1.42 kg), with highest R^2C (0.47) and R^2P (0.40) (Table 2). Our prediction accuracy of NIRS for WBSF were close to found

Table 2. Calibration statistics for the determination of meat quality traits by NIRS

Trait ¹	Preprocessing technique ²	RMSEC ³	RMSEP ³	R ² C ⁴	R ² P ⁴
WBSF	RAW	1.44	1.53	0.38	0.31
	RSR	1.34	1.42	0.47	0.40
	MSC	1.51	1.60	0.32	0.24
	MSC + RSR	1.37	1.47	0.44	0.36
	1D	1.80	1.84	0.04	0.00
	1D + RSR	1.44	1.58	0.38	0.26
	MSC + 1D	1.80	1.84	0.04	0.00
	MSC + 1D + RSR	1.40	1.58	0.41	0.26
Marbling	RAW	0.15	0.15	0.00	0.00
	RSR	0.15	0.15	0.00	0.00
	MSC	0.15	0.15	0.01	0.00
	MSC + RSR	0.15	0.15	0.01	0.00
	1D	0.15	0.15	0.01	0.00
	1D + RSR	0.15	0.15	0.03	0.01
	MSC + 1D	0.15	0.16	0.02	0.00
	MSC + 1D + RSR	0.15	0.15	0.04	0.02
a*	RAW	1.40	1.43	0.20	0.16
	RSR	1.40	1.42	0.20	0.17
	MSC	1.47	1.49	0.11	0.10
	MSC + RSR	1.47	1.48	0.11	0.10
	1D	1.54	1.58	0.03	0.00
	1D + RSR	1.52	1.53	0.05	0.04
	MSC + 1D	1.54	1.58	0.02	0.00
	MSC + 1D + RSR	1.53	1.54	0.04	0.02
b*	RAW	1.54	1.97	0.49	0.45
	RSR	1.89	1.21	0.46	0.44
	MSC	1.26	1.33	0.39	0.32
	MSC + RSR	1.27	1.30	0.39	0.36
	1D	1.48	1.55	0.16	0.08
	1D + RSR	1.21	1.34	0.44	0.31
	MSC + 1D	1.51	1.58	0.12	0.04
	MSC + 1D + RSR	1.21	1.31	0.44	0.34
L*	RAW	1.92	1.98	0.21	0.16
	RSR	1.95	1.98	0.19	0.16
	MSC	2.09	2.12	0.06	0.04
	MSC + RSR	2.01	2.07	0.14	0.09
	1D	2.13	2.18	0.03	0.00
	1D + RSR	2.12	2.14	0.04	0.02
	MSC + 1D	2.13	2.19	0.02	0.00
	MSC + 1D + RSR	2.12	2.14	0.04	0.02

¹WBSF = Warner-Bratzler shear force; a* = redness; b* = yellowness; L* = lightness.

²RAW = raw data; RSR = reduction of spectral range; MSC = multiplicative scatter correction; 1D = 1st order derivative.

³RMSEC and RMSEP: root mean square error of calibration and prediction.

⁴R²C and R²P: determination coefficient of calibration and prediction.

by several authors (Rødbotten et al., 2000; Leroy et al., 2003; Prieto et al., 2008; Cecchinato et al., 2011; De Marchi et al., 2013) and they were worse than those reported by Park et al. (1998) and Prieto et al. (2014). It is important to note that all studies cited here to compare our results used different approaches, i.e., spectra measured in the reflectance and transmittance mode or in different wavelength ranges, type of meat used, and different instruments

were used for spectrum collection. For example, Prieto et al. (2014) used ground beef for spectrum collection and a wider wavelength range (400 to 2498 nm), while De Marchi et al. (2013) used both, ground and intact beef, with spectroscopy of visible, NIRS and near-infrared transmittance. These authors reported that the type of sample used for infrared scanning was crucial for the prediction of WBSF, with better predictions of WBSF in intact

compared with homogenized samples. According to Liu et al. (2003), direct comparison of the prediction results for meat quality is difficult because of the different conditions of meat, with samples varying in the state of freshness, aging, frozenness, and thawing. Nevertheless, most studies generally reported a moderate prediction ability of NIRS for WBSF, probably because this trait was measured by physical analysis, while NIRS is based on the radiation absorbed by chemical bonds. Thus, NIRS is less reliable for predicting physical meat quality traits than chemical analyses (Prevotnik et al., 2004).

Among all traits studied, the lowest prediction ability of NIRS was observed for marbling. Data regarding the ability of NIRS to predict marbling are limited in cattle because most studies have used chemical analysis to measure the percentage of intramuscular fat. Chan et al. (2002) used NIRS to predict marbling scores in swine and reported an R^2 of 0.35, which was higher than the 0.02 found here (Table 2). Two problems may explain the inefficiency of NIRS to predict marbling. The first refers to the reference analysis of marbling, which is a subjective trait and scores are therefore used for its evaluation, which increases the chance of error. To overcome this problem, a more precise and reliable measurement, such as extractable lipid should be used instead of marbling score. In addition, as can be seen in Table 1, the standard deviation and coefficient of variation were low, therefore being difficult to use NIRS to predict traits with small variation. The second problem might be related to the beef used for spectrum collection. We used intact meat samples which, according to Rødbotten et al. (2000), have a heterogeneous distribution of fat. Thus, homogeneous mixtures such as ground or minced meat would be more adequate to collect NIR spectra since they increase the chance of detecting the “true” composition of fat. Furthermore, the small variation among the samples (standard deviation of 0.15) most likely decreased the predictive performance and robustness of the calibration model, generating a low R^2 .

Regarding color traits, higher prediction accuracies were obtained for b^* and lower accuracies for a^* and L^* (Table 2). The prediction accuracies for the color traits were lower than those reported in other studies (Liu et al., 2003; Prieto et al., 2014). This difference in the results found for these traits may be related to the spectral region used for prediction in this study (2,500 to 1,000 nm). This NIR region is associated with high energy vibrational transitions related to the vibration of chemical bonds and not with electronic transitions observed in the visible

range. According to Van Den Oord and Wesdorp (1971), the visible spectrum (630 and 580 nm) is associated with the percentage of oxymyoglobin, one of the forms of myoglobin (pigments that determine meat color). In addition to the spectral range, another factor that could explain the low prediction accuracy of meat color is the time elapsed between the objective color measurements and NIRS analysis (Prieto et al., 2014). In the present study, this interval was not measured but was probably not the same for all traits, which could explain the low accuracy obtained for the L^* and a^* parameters. According to Prieto et al. (2014), the time elapsed between spectrum collection and reference analysis can modify the oxidation states of the myoglobin pigments in meat, thus giving rise to changes in color.

CONCLUSIONS

NIRS showed a better ability to predict WBSF and b^* color when RSR in the range of 4,132 to 9,112 cm^{-1} was used as preprocessing technique, while its ability to accurately predict L^* and a^* was limited. The ability of NIRS to predict subjective meat quality traits such as marbling in Nelore cattle is low because of the presence of heterogeneities in the meat samples, low precision of the reference analysis, and low variation between samples.

NIRS is a useful tool to be applied in the meat industry, predicting the traits of tenderness and meat color. However, NIRS should not be applied to predict subjective meat quality traits. Reference analysis to analyze beef fat should be improved in order to evaluate the ability of NIRS to predict fat content in bovines.

Conflict of interest statement. The authors declare that they have no conflict of interest.

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