Evaluation of Alternative Sample Preparation Methods for Development of NIR Models to Assess Chemical Properties of Wood

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The aim of this work was to investigate the influence of sample preparation including variation in moisture content and particle size on the accuracy of near infrared (NIR) spectroscopy models developed to predict Klason lignin, total lignin, and holocellulose in wood. Seventy-five samples of sawdust obtained from a eucalyptus plantation were divided into aliquots and submitted to three different treatments: traditional (TRAD), large particle dried at room temperature (LPRT), and large particle oven-dried (LPOD). The influence of sample preparation method on models' accuracy was compared by statistical analysis. Overall, grinding to a larger particle size and drying at room temperature (treatment LPRT) did not decrease the accuracy of the prediction models when compared to the TRAD sample preparation method. These findings were more evident for Klason lignin and holocellulose. This is relevant because resources used for sample preparation (*i.e.* grinding and drying) can be minimized, which is expected to reduce the costs associated with analysis of wood properties by NIR.

Keywords: Holocellulose; Lignin; Near infrared (NIR) spectroscopy; Eucalyptus wood; Particle size

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INTRODUCTION

Near infrared (NIR) spectroscopy is a successful scientific tool for characterizing many biomaterials, including wood (Tsuchikawa 2007; Pažitný *et al.* 2011; Tsuchikawa and Schwanninger 2013). The number of publications reporting NIR spectroscopy application to forestry, wood, and wood products is increasing systematically (Tsuchikawa and Kobori 2015; Sandak *et al.* 2016). These applications include determination of wood quality and pulp characteristics (Birkett and Gambino 1989; Castillo *et al.* 2012), prediction of lignin syringyl/guaiacyl content (Hein *et al.* 2010; Lupoi *et al.* 2014; Ramadevi *et al.* 2016), and the prediction of ashes, extractives, lignin, holocellulose, and wood cellulose content (Wright *et al.* 1990; He and Hu 2013; Zhou *et al.* 2015, 2016). Additional uses include characterization of anatomical, physical, and mechanical properties of wood (Hein 2012; Milagres *et al.* 2013; Sundaram *et al.* 2015); wood bioenergy (Fagan *et al.* 2011; Hou and Li 2011; Ramalho *et al.* 2017), and discrimination of similar rare woods (Yang *et al.* 2012; Shou *et al.* 2014).

NIR is a rapid, nondestructive, and safe method for determining the variability of many wood traits from a single sample. Providing a quick and reliable quantitative estimation of Klason lignin, holocellulose, and α-cellulose content can improve the efficiency of feedstock processing in industrial environments. Additionally, NIR provides great advantages for plant germplasm screening, genetic engineering, and industrial crop cultivation.

The success of NIR-based analyses is driven by many factors such as understanding how instrument parameters and sample conditions affect the spectral signatures, as well as how to extract useful information from the spectra. Wood processing before analysis is a key factor because significant variability in prediction may be introduced by the sample preparation method (Hein *et al.* 2010). For example, moisture content that is not uniform among samples can introduce an undesirable source of variation. Conversely, care must be taken when drying samples in order to avoid potential sample damage or changes in the chemical structure of the wood (Sandak *et al.* 2016).

The influence of sample moisture content on the NIR spectra has been described previously (Tsuchikawa and Tsutsumi 1998; Thygesen and Lundqvist 2000a, b; Watanabe *et al.* 2006; Schwanninger *et al.* 2011). Overall, studies suggest that the samples can be oven-dried to decrease moisture content before being read by the NIR (Lupoi *et al.* 2014). Because water is a strong absorber in the NIR region, samples with high moisture are strongly dominated by the signature from water. However, it remains unknown whether different temperatures and sample moisture content influence the statistics generated with the prediction models developed from those spectra.

Wood preparation before analysis is normally based on mechanical treatment that produces wood powder. TAPPI T257 CM-02 (2002) recommends grinding wood to a particle size that passes a 40 mesh (0.4 mm) screen, whereas heating the material during grinding or regrinding the material should be avoided. However, the resulting particle size may be too large for NIR spectroscopy analysis, and it then becomes necessary to regrind or fractionate the sample (Schwanninger *et al.* 2004). Additionally, it is known from TAPPI T264 CM-07 (2007) that different fractions may contain varying quantities of some wood constituents, so that their removal could modify the chemical composition of the sample. Similarly, regrinding can also significantly modify the chemical composition of the wood material.

This study evaluated the effects of sample preparation (*i.e.*, variation in moisture content and particle size) for NIR spectra acquisition on the accuracy of multivariate models for predicting Klason lignin, total lignin, and holocellulose associated with wood quality. While the effects of moisture content and particle size on NIR spectral determination have been previously described, this research attempted to find a less tedious sample preparation method for estimation of chemical components of wood. If costs associated with sample preparation can be minimized (*i.e.*, grinding and drying) without compromising model accuracy, then the costs associated with NIR analysis can be reduced.

EXPERIMENTAL

Materials

The wood material used came from a *Eucalyptus benthamii* progeny test plot installed in 2011 in the municipality of Encruzilhada do Sul, in the state of Rio Grande do Sul, Brazil (S -30.65268 e W -52.46839). The test plot area was 1.3 ha, and the spacing between trees was 3.50 m by 2.14 m, which allowed a total of 1394 trees in the experiment. Samples for the study were taken 55 months after planting the trees.

Methods

Selection of trees and sample collection

An initial sample was collected from all 1394 trees using a non-destructive method. This procedure included using a drill equipped with a special bit to collect core sawdust from four spots at the diameter of breast height, or 130 cm from the ground. The sawdust was processed into smaller particles using a Willye mill equipped with a 1 mm sieve. The portion of the sample that passed through the 1 mm sieve was analyzed using the NIR. Based on the spectra, the variable pulp yield and the syringyl/guaiacyl (S/G) ratio were estimated using calibration models developed for *Eucalyptus globulus* and *Eucalyptus grandis* (Alves *et al.* 2011a, 2012). Subsequently, the estimates of pulp yield and S/G ratio from the 1394 trees were used to select 75 representative *Eucalyptus benthamii* trees for the development of predictive models based on NIR spectroscopy. The selected subgroup included 20 trees with high pulp yield (55% to 62%), 20 with low pulp yield (36% to 43%), and 25 with intermediate values (44% to 54%). Additionally, 5 trees were selected to represent high S/G value (1.70 to 2.10) and 5 to represent low S/G (1.10 to 1.69).

A log of about 70 cm was collected immediately above the diameter of breast height (approximately 130 cm from the ground) from all 75 trees. This log was then chopped into chips. An aliquot of this material was used for standard wet chemistry tests, and another portion was used for measurements with the NIR as described below.

Wet chemistry analysis

Chips without bark and knots were classified following SCAN CM-40:01 (2001). The accepted fraction was ground in a knife mill and sieved according to TAPPI standard T257 CM-02 (2002), where the fraction that passed through the 40 mesh (40 openings per mm^2 or size of the opening equal to 0.47 mm) and retained in the 60 mesh (0.31 mm) was used for analysis. Subsequently, the chemical properties of Klason lignin, total lignin, and holocellulose were determined by wet analysis chemistry and served as the dependent variables for which the calibration models in the NIR were adjusted against. The standards used in the wet analysis chemistry were TAPPI T222 OM-2 (2002), TAPPI UM-250 (1991), and TAPPI T203 CM-99 (1999), for Klason lignin, total lignin, and holocellulose, respectively. All analyses were performed on a dry matter basis.

Treatments and Fourier transform near-infrared (FT-NIR) acquisition

The aliquot of sawdust designed for NIR analysis was initially processed into smaller particles in a Willye mill equipped with a 1 mm sieve. A fraction of the material that passed through this sieve was collected and split into 3 aliquots for application of the treatments. There were three treatments: traditional (TRAD), large particle dried at room temperature (LPRT), and large particle oven-dried (LPOD). Descriptions of the three treatments, including drying method and particle size, are provided in Table 1. Briefly, TRAD treatment is the protocol commonly used for NIR analysis. The LPRT and LPOD treatments are sample preparation methods that reduce sample grinding and facilitate the drying process.

After the treatments were applied, diffuse reflectance spectra were acquired using a Fourier transform NIR (FT-NIR) spectrometer (Model: MPA, BrukerOptik GmbH, Ettlingen, Germany). The spectra were read between 12000 cm^{-1} and 4000 cm^{-1} with a resolution of 8 cm^{-1} and used to develop prediction models for Klason lignin, total lignin, and holocellulose.

Table 1. Description of the Three Treatments (*i.e.*, Sample Preparation Method) Applied before NIR Spectra Acquisition

	TRAD	LPRT	LPOD
Drying Temperature (°C)[1]	22	22	60
Drying Time (h)	24	24	48
Final Moisture content (%)	12	12	7
Particle size (mm[2])	0.47-0.31	1	1

[1] Drying methods were done in a room temperature (22 °C) or forced-air oven drying (60 °C)
[2] Material that passed through the 1mm sieve and material that passed through the 0.47 mm sieve (40 mesh) but was retained in the 0.31 mm sieve (60 mesh).

Multivariate statistics and data analysis

Calibrations were performed using the Bruker Opus 6.2 software package. In the same software all spectra were pre-processed with four different procedures: 1) first derivative (1stDer); 2) vector normalization (VN); 3) first derivative + vector normalization (1stDer + VN); and 4) first derivative + a multiplicative correction of the signal (1stDer + MSC). Optimization of Savitzky-Golay (SG) smoothing was used for spectroscopy analysis.

Model calibration was determined by regression analysis of the partial least squares (PLS), as recommended by Gierlinger *et al*. (2002). The models for the treatments were adjusted with the same number of latent variables (LVs) needed to provide the best fit. The number of variables adopted for each model was considered as a function of the decrease of the root mean square error of cross-validation (RMSECV) and the increase of the coefficient of determination (R^2) and residual predictive deviation (RPD).

Spectral bands above 10000 cm^{-1} were excluded because in this region the spectrum presented noise repetitions that did not contain relevant information about the properties of interest. The spectral ranges used for construction of calibration models were based on the recommendations of Schwanninger *et al.* (2011). The anomalous samples visibly different from the normal distribution were detected as outliers and excluded from the model.

Calibration models were developed for Klason lignin, total lignin, and holocellulose using all 75 observations in each treatment. Subsequently, these models were run in 15 different regions of the spectra to generate replications that allowed statistical testing of model accuracy. A statistical analysis was applied to verify the difference between the mean values of the validation error (RMSECV) of the models using the statistical software Genes (Cruz 2001). When the null hypothesis was rejected, a comparison of means was done by means of a Tukey test (5%) and data compliance was verified through the error normality tests (Shapiro Wilks).

RESULTS AND DISCUSSION

Wet Chemistry

The descriptive statistics of chemical components observed in *Eucalyptus benthamii* are presented in Table 2. The mean values of Klason lignin, total lignin, and holocellulose found in wood are in agreement with other species of the genus previously studied at similar age (Estopa *et al*. 2017). Total lignin content was 31.3%, which is lower than what was reported by Trugilho *et al*. (2015) in *Eucalyptus cloeziana* (32.3%) and higher than what was determined for 75 *Eucalyptus* clones (27.3%) commercially used in Brazil (Gomide *et al*. 2010). Klason lignin averaged 27.7% and was higher than what was previously reported for *E. benthamii* (26.2%, Alves *et al*. 2011b), and for *E. urophylla* × *E. grandis* (26.9%, Baillères *et al*. 2002).

Table 2. Descriptive Statistics of Chemical Components of *Eucalyptus benthamii*

Chemical component	Mean (%)	Range (%)	SD (%)	N[1]
Klason Lignin	27.7	24.6 to 30.7	1.3	75
Total Lignin	31.3	28.1 to 34.4	1.2	75
Holocellulose	64.8	56.2 to 68.2	1.8	75
[1] Number of samples				

Holocellulose content averaged 64.8%, which is lower than in hardwood trees (79.6%, Zhou *et al*. 2015), but higher than the value reported by Trugilho *et al*. (2015) for *Eucalyptus urophylla* x *Eucalyptus grandis* (59.7%). Of note, *E. urograndis* is the hybrid that is planted the most in Brazil.

In most *Eucalyptus* species the lignin content decreases as the tree develops and forms the adult wood. In contrast, the content of carbohydrates tends to increase as time progresses (Trugilho *et al*. 1996; Estopa *et al*. 2017).

Although the determination of Klason lignin, total lignin, and holocellulose was relevant for comparison of the dataset against published studies, the main goal of the wet chemistry analysis was to produce reference data for the NIR. The authors observed considerable variation in the dataset and ensured accuracy of the reference methods, which are the two most important factors for calibration and development of accurate NIR models (Alves *et al*. 2011a).

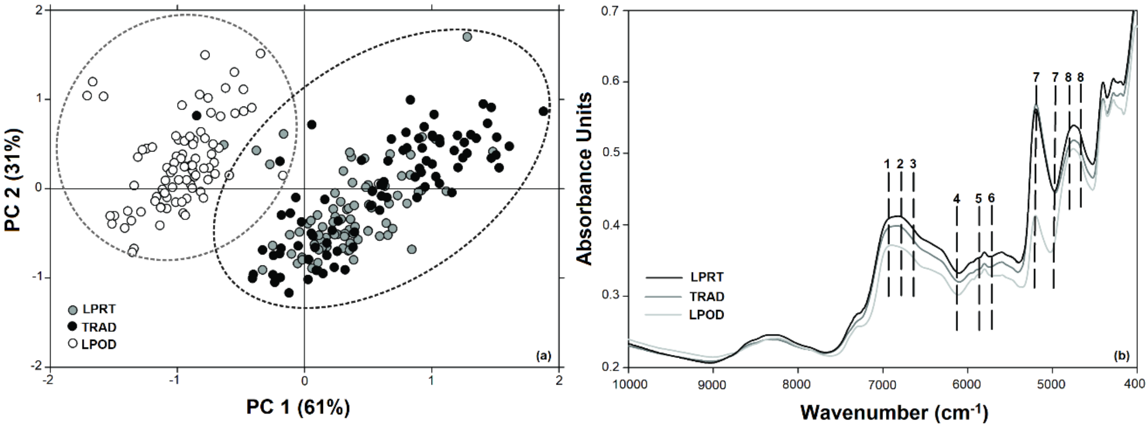

Fig. 1. a) Score plot obtained by PCA applied to NIR spectra collected from the three different treatments; b) Absorbance versus wavenumber plot for untreated average NIR spectra of the three treatments. Bands assigned to chemical compounds are represented by numbers and listed in Table 3

Effect of Treatments on the Spectra

The PCA applied to the FT-NIR spectra highlighted the discriminating ability of the NIR to separate samples by plotting the averaged spectra of 75 samples for each treatment (Fig. 1a). The PCA scores plot of spectra acquired from the wood specimens shows at least two groups (indicated by dotted circles). The two main components together explain 92% of the variability of the data analyzed, of which 61% is explained by the Principal Component 1 (PC1) and 31% is explained by Principal Component 2 (PC2).

The differences in the spectra due to treatment effect were assessed by plotting and overlapping the average of the 75 samples for each treatment (Fig. 1b). The reference for the NIR region interpretation is based on Watanabe *et al*. (2006), Schawninger *et al*. (2011), and Sandak *et al*. (2011) (Table 3).

Overall, the three spectra showed strong absorption bands. This was due to the overtone of the OH stretching vibration at 7200 cm^{-1} to 6000 cm^{-1} and the combination of OH stretching and deformation vibrations at 5400 cm^{-1} to 4800 cm^{-1} and 4900 cm^{-1} to 4600 cm^{-1}. The spectra of the three bands increased progressively, with LPRT having the highest peak, followed by TRAD and LPOD (Fig. 1b). The lowest absorbance peaks were seen in the spectra recorded from samples with the lowest moisture content (LPOD treatment). This observation agrees with results published by Inagaki *et al*. (2008). Variation in the NIR spectra due to sample moisture content was observed throughout the NIR range, which indicated that the absorption peaks due to OH bonding of water were substantially overlapped in the original spectra. This variation in absorbance due to moisture content is governed by the adsorption/desorption mechanism, where the wood–water interaction or the physicochemical properties of water play an important role. Because the NIR light absorption by wood overlapped with that of water, the spectroscopic information was compromised. Importantly, the mechanism of which moisture content in wood influences the acquisition and behavior of NIR spectra has been extensively discussed previously (Tsuchikawa and Tsutsumi 1998; Thygesen and Lundqvist 2000a, b; Watanabe *et al*. 2006; Schwanninger *et al*. 2011; Pecoraro *et al*. 2015).

Table 3. NIR Absorption Bands Associated with Components Present in Wood Specimens. Index Numbers Related to the Specifics Bands Indicated in Fig. 1.

Index	Wavenumber (cm^{-1})	Bond Vibration	Structure	Ref.[1]
1	≅6913	OH stretching first overtone	Phenolic OH group	1
2	6800	OH stretching first overtone	Glucomannan	2
3	6775	OH stretching first overtone	Semi-crystalline region in cellulose	2
4	6281	OH stretching first overtone	Crystalline region in cellulose	2
5	5935	CH stretching first overtone	Aromatic skeletal in lignin	3
6	5800	CH stretching of first overtone	Furanose or pyranose in hemicelluloses	1
7	5220 and 5051	OH stretching plus OH deformation	Water	2
8	4780 and 4762	OH and CH deformation plus OH stretching	Cellulose and xylan	2

[1] References consulted: 1 - Watanabe *et al*. (2006); 2 - Schwanninger *et al*. (2011); 3 - Sandak *et al*. (2011)

The principal component analysis grouped LPOD treatment in a separate cluster and combined LPRT and TRAD treatments in another (Fig. 1a). In LPOD treatment, the dried wood showed only hygroscopic water, and the water band was expressed with less intensity at the range of approximately 5200 cm^{-1} (combination of stretching and deformation vibrations for OH). However, the location of the band can be modified with increased moisture content in the wood, with NIR absorption also apparent at 5000 cm^{-1}, 5080 cm^{-1}, and 4920 cm^{-1} (Schwanninger *et al*. 2011). From a different prospective, analyzing the NIR spectra could be a tool to screen the content and the state of water in unknown samples (Tsuchikawa and Tsutsumi 1998; Terazawa *et al.* 2003; Inagaki *et al.* 2008). It should be noted that the shapes of the bands were similar among treatments (Fig. 1b), and the most conspicuous changes in the spectra of treated wood were only observed when analyzing the wavenumbers (Table 3).

Overall, the spectral absorption was more influenced by sample moisture content than particle size (Fig. 1b). The greatest absorbance values were evidenced in the TRAD and LPRT treatments, which had greater moisture content compared with LPOD (12.0% *vs*. 7.0%). This result is somewhat expected because water has great capacity to absorb electromagnetic radiation (Achata *et al*. 2015). Therefore, the results suggested that when preparing wood samples for NIR analysis, controlling sample moisture content might be more important than trying to grind samples to a particle size smaller than 1 mm. Thus, LPOD may be preferred particularly for preparation of a large number of samples. It should be noted that alternative methods that minimize the drying time should be studied.

Differences between NIR spectra recorded from 0.5 mm or 4.0 mm particle size appear to be less significant with absorbance variation continuously similar along the NIR spectral range (Hein *et al*. 2010). These same authors mentioned that the ball milling process resulted in more homogeneous spectra when compared to solid wood particles (*e.g.* wood chips).

This was not surprising, since the band at 1400 nm of the fine powder was stronger than that of the raw wood meal. The more intensive the ball milling, the higher the percentage of fine material in the sample, and this explains the increase in absorbance values in some spectra regions (Schwanninger *et al*. 2004). However, ball milling is a time-consuming laboratorial procedure that can preclude processing of a large number of samples. Variations in particle size between 30 mesh and 60 mesh of *Eucalyptus* wood did not have a significant effect on the NIR spectra (Baillères *et al*. 2002). Nevertheless, in *Eucalyptus globulus* the NIR-estimated lignin content was influenced by particle size (Poke and Raymond 2006).

Calibration Models

The partial least square regression models for chemical components predicted by NIR spectroscopy for each treatment are presented in Table 4. The Klason lignin models presented promising statistics and showed potential for providing a method of accessing quality control for individual samples (Figs. 2a to 2c). Predicted and observed values for Klason lignin contents exhibited good correlations with R^2 of 0.87, 0.90, and 0.86 for LPRT, LPOD, and TRAD treatments, respectively. The RPD values of validations were lower than 3, indicating that such models may be adequate for screenings or for genetic studies (Schimleck *et al*. 2003).

Table 4. Cross-validation Statistics of Partial Least Square Regression Models for Calibration of Chemical Components in *Eucalyptus benthamii*

Treatment	Component	PreProRange	VL	R²cv	RMSECV	RPD	Outliers
	Klason lignin	3 (d+j)	5	0.86	0.51	2.75	12
TRAD	Total Lignin	1 (d+j)	4	0.80	0.59	2.25	9
	Holocellulose	2f	6	0.81	0.72	2.34	6
	Klason lignin	2g	8	0.87	0.49	2.85	14
LPRT	Total Lignin	3(c+i)	6	0.78	0.66	2.14	7
	Holocellulose	3 (c+i)	6	0.84	0.69	2.52	11
	Klason lignin	2 (a+d)	3	0.90	0.46	3.17	13
LPOD	Total Lignin	1h	4	0.82	0.61	2.39	8
	Holocellulose	1(b+i)	5	0.81	0.75	2.31	8

Where 1stDer: First Derivative, VN: Vector Normalization, PreProRange: pre-processing treatment and wavenumber range (cm^{-1}); VL: Average number of factors used in model construction; R²cv: Coefficient of Determination for Cross-Validation.; RMSECV: Root Mean Standard Error of Cross-Validation; RPD: Residual predictive deviation; Outliers: Number of outliers removed from calibration models.

1 – 1stDer a – 9403.7-7498.2 cm^{-1}; b – 9403.7-6098.1 cm^{-1}; c – 7502.1-6098 cm^{-1};
2 – 1stDer+VN d – 6102-5446.3 cm^{-1} e – 6102-5446 cm^{-1}; f – 6102-4597.7 cm^{-1};
3 – VN g – 6101.9-4246.7 cm^{-1}; h – 5176.3-4246.7 cm^{-1}; i – 5450.1-4246.7 cm^{-1};
j – 4601.5-4246.7 cm^{-1}

Overall, values of R^2 for total lignin and holocellulose can be considered acceptable (*i.e.* 0.82 in LPOD and 0.84 in LPRT), and the RMSECV values were below 0.61% and 0.69%, respectively (Table 4, Figs. 2f and 2h). Validation of these calibrations produced good correlations between wet chemistry predicted values in the laboratory and NIR-predicted values. Other researchers have published calibration models for total lignin in *Eucalyptus* trees. However, these models showed lower R² (0.71) and higher RMSECV (0.90%) (Milagres *et al.* 2013). A better calibration (R² = 0.78) was developed using NIR analysis for predicting Klason lignin content from solid wood in *Eucalyptus globulus* (Poke and Raymond 2006). For holocellulose, Hou and Li (2011) developed a model containing 8 PLS vectors, which resulted in high R² (0.98) and low RMSECV (0.34%).

With regard to the lignin content in *Eucalyptus benthamii* wood, calibration models with coefficient of determination of 0.64 and RMSECV of 0.68% have been published (Estopa *et al.* 2017). The present study yielded excellent cross-validation statistics for lignin content, with higher coefficient of determination and lower RMSECV for all treatments (Table 4).

Comparison of PLS-R Models

An important goal of this study was to evaluate differences between the calibration models related to different sample preparation protocols prior to collecting the spectra. It was anticipated that if no difference between treatments was evident, then the sample preparation work can be minimized, which reduces costs and increases the speed of NIR analysis.

When a clear decision cannot be made regarding the choice of the best model, more rigorous examination of the results is required. Examining the cross-validation of the models resulting from the combination of multiple data sets could help in making such a decision (Alves *et al.* 2011a). Alternatively, statistics such as comparison of means can be applied to differentiate the values of RMSECV.

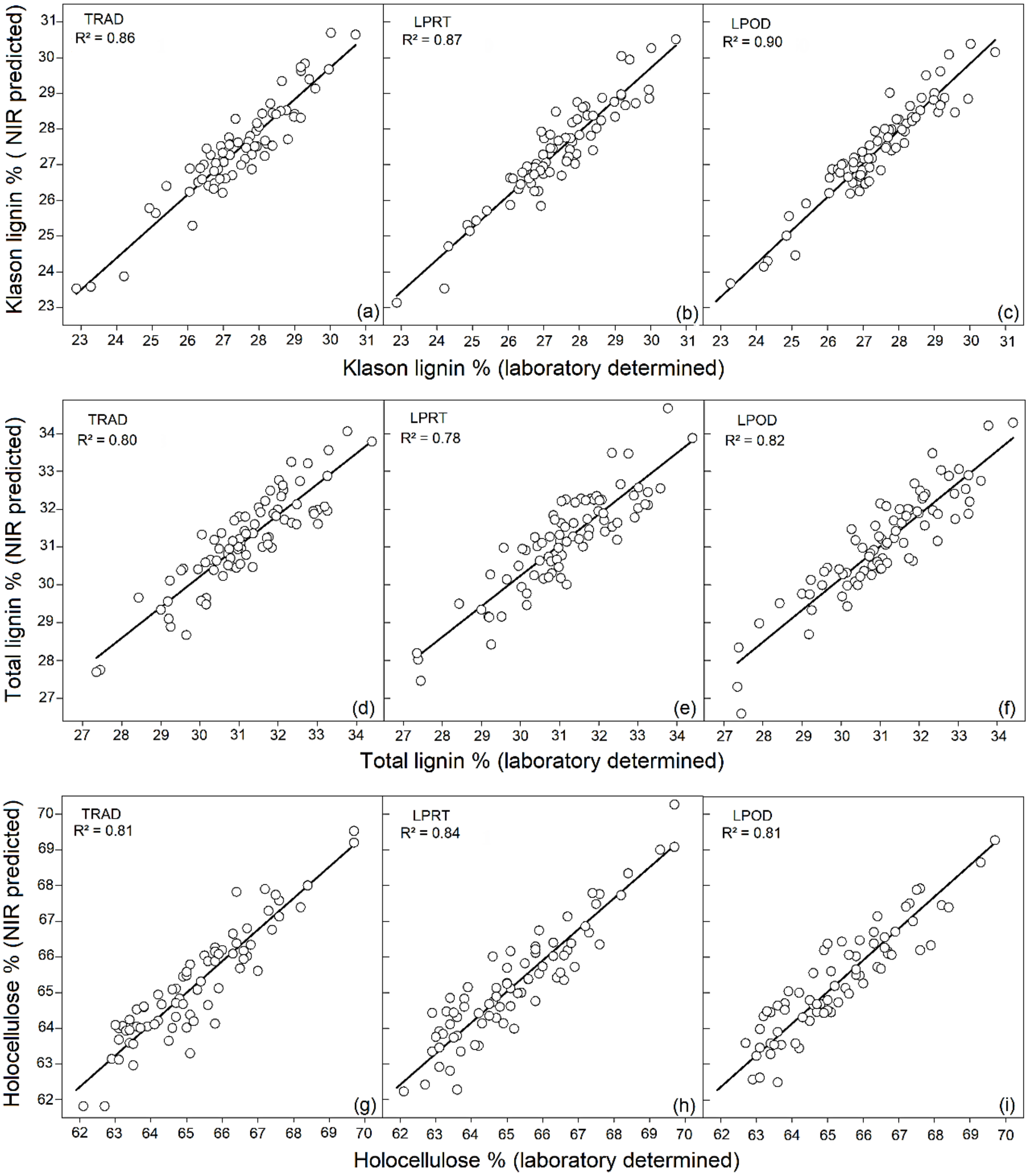

Fig. 2. Cross-validation predictions of Klason lignin, total lignin, and holocellulose in *Eucalyptus benthamii*

The mean comparison test applied on the 15 RMSECV values of the calibration models for each treatment is presented in Fig. 3. Regarding Klason lignin, LPOD treatment yielded satisfactory prediction and was not statistically different than TRAD treatment (Fig. 3a). Additionally, no difference was observed between the LPOD and LPRT treatments. This finding indicated that for measurement of Klason lignin, samples do not need to be ground to a particle size smaller than 1 mm or reduced to a moisture content smaller than 12%. This is relevant as it substantially reduces sample preparation time and labor cost.

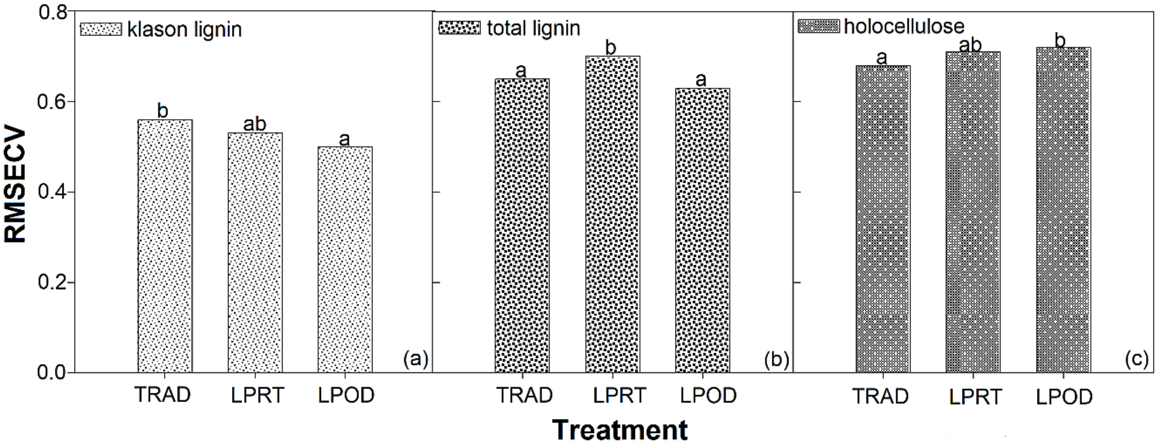

Fig. 3. Treatment effect on RMSECV values for calibration models of Klason lignin (a); total lignin (b); and holocellulose (c)
* Means followed by the same letter in the column do not differ by Tukey test at 5% probability

With regard to total lignin, the model error in LPOD and TRAD treatments was smaller than that in LPRT treatment (Fig. 3b). This indicated that, for analyses of total lignin, samples should be ground to particles sizes less than 1 mm or, if grinding to 1 mm, sample moisture content should be reduced to less than 12%. A noteworthy observation was that LPOD treatment, which had samples with lower moisture content, presented the overall best statistics for total lignin model fit. This was mainly because the bands related to water adsorption were excluded from the calibration models. Moisture content was important for selecting the best models for total lignin, and was also an indicator of dryness of wood particles after sample preparation. For holocellulose, TRAD treatment produced better predictions than LPOD treatment, but did not differ from LPRT (Fig. 3c). This indicated that in addition to measurements of Klason lignin, LPRT treatment can be applied on samples when analyzing holocellulose.

NIR analysis has commonly been performed using the fraction of the wood milled through the 40 mesh to 60 mesh, particularly because this is the fraction used for gold standard wet chemistry analyses. However, this study demonstrated that accurate NIR models could be developed using samples of larger particle size (*i.e.* 1 mm, LPRT and LPOD treatments). Furthermore, except for the determination of total lignin that required either smaller particle size (TRAD treatment) or lower sample moisture content (LPOD treatment), this study demonstrated that samples of larger particle size (1 mm) can be dried at room temperature. The authors expected that the alternative sample preparation methods evaluated in this study could be applied to wood samples other than *Eucalyptus*. However, special attention should be given to sample final moisture content because of the disturbing effects of water on the quality of the NIR spectra. Additionally, further research is warranted using similar sample preparation methods, particularly on wood samples that expand the range of Klason lignin, total lignin, and holocellulose outside the boundaries evaluated in this study.

CONCLUSIONS

1. This study demonstrated that sample preparation work can be minimized (*i.e.*, grinding to 1 mm and drying at room temperature), which can considerably reduce the costs associated with NIR analysis.
2. Compared with the traditional sample preparation method, grinding to a larger particle size and drying at room temperature (LPRT treatment) did not affect the accuracy of the prediction models, particularly for Klason lignin and holocellulose. For analysis of total lignin, samples can be ground to a larger particle size, but the quality of the calibration model was only equivalent to the traditional method when moisture content was reduced to 7% (LPOD).
3. Promising NIR models were developed for predicting chemical components (*i.e.*, total lignin, Klason lignin, and holocellulose) associated with wood quality. Statistical analysis of the error associated with the developed NIR models demonstrated minor differences between the treatments.

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