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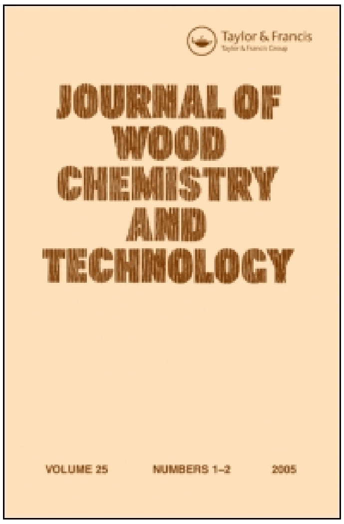

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Abstract: Near-infrared (NIR) spectroscopy provides a rapid method for estimating pulp carbohydrate content. Previous studies have used a variety of sample preparation methods; however, the influence of preparation method on calibration performance has not been examined. This study compares carbohydrate content calibrations for four sample preparation methods (coarse, fine, and milled pulp, and handsheets). Fifty-nine unbleached pulps (52 single-tree, 7 mill composite) with known carbohydrate composition (arabinose, fructose, galactose, glucose, rhamnose, and xylose) were prepared using the four methods. NIR spectra were collected from the samples, which were divided into calibration (40 samples) and prediction sets (19 samples). Calibrations were created for each combination of preparation method and carbohydrate, and tested on the prediction set. Calibration statistics were good for rhamnose and xylose but weaker for other carbohydrates. Coarse and fine pulp produced the best calibrations, but they were not significantly different from calibrations for handsheets; milled pulp provided the weakest calibrations.

Keywords: Carbohydrate composition, near infrared spectroscopy, sample preparation, unbleached eucalyptus pulp

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INTRODUCTION

Near-infrared (NIR) spectroscopy is a tool that is being used increasingly in the pulp and paper industry to rapidly estimate a variety of wood and pulp properties. One possible use of considerable interest is the measurement of the carbohydrate composition of pulp. Wood pulps comprise a variety of different carbohydrates, including arabinose, fructose, galactose, glucose, mannose, rhamnose, and xylose. The relative proportions of these carbohydrates vary widely among the major pulpwood species, but glucose and xylose generally make up the greatest and second-greatest percentages, respectively, of total carbohydrates.

Carbohydrate composition affects many significant physical and mechanical properties of pulp and paper. Hemicelluloses are very important to interfiber bonding allowing cellulose fibers to swell, which improves burst and tensile strength of papers.[1] However, hemicelluloses, particularly xylose, can also have a deleterious effect on paper if the brightness of the paper is of primary concern. Xylose binds cellulose and lignin together, so the elimination of xylose through the use of xylanases allows the lignin to be more readily eliminated in subsequent bleaching stages.[2,3]

For many years, the primary method of pulp carbohydrate composition determination has been acid hydrolysis, which requires severe conditions and laborious procedures.[4] More recently, pyrolysis gas chromatography has proven to be an effective alternative to acid hydrolysis. In this procedure, a pulp sample is quickly pyrolyzed in an inert atmosphere, and the resultant products are analyzed by gas chromatography.[5] Although it is accurate and more user-friendly than acid hydrolysis, pyrolysis gas chromatography requires very expensive equipment.

NIR spectroscopy may provide a more rapid and inexpensive approach for estimating carbohydrate composition. Wallbäcks et al.[6] investigated the use of spectroscopic methods to predict the chemical compositions, that is, carbohydrate composition and lignin content, of unbleached Kraft pulps. They found that glucose, xylose, and lignin, the primary constituents of the pulps, were well-modeled. Of the remaining pulp constituents (arabinose, galactose, and mannose) only galactose was well-modeled. Another study from the same year focused on the use of NIR spectroscopy to predict many physical and chemical properties of bleached pine pulps, including arabinose, galactose, glucose, mannose, and xylose content.[7] The study examined a small number of samples and only reported predictions for glucose and mannose, but it clearly demonstrated the potential of NIR to predict carbohydrate compositions of various pulps.

Although many subsequent studies have investigated the use of NIR spectroscopy to predict the lignin content or kappa numbers of pulps, carbohydrate composition has been largely ignored. An exception is a study published by Fardim et al.[8] that examined the potential for NIR to predict numerous

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properties of unbleached kraft *Eucalyptus grandis* pulps, including carbohydrate composition, uronic acids, lignin content, Kappa number, relative viscosity, and Inernational Organization for Standardization (ISO) brightness. The carbohydrates that they determined for the study were arabinose, galactose, glucose, mannose, rhamnose, and xylose. However, of these six carbohydrates, only glucose and xylose content were analyzed using NIR spectroscopy. They found that both glucose and xylose were well-modeled using four and three partial least squares (PLS) factors, respectively, but the standard errors of cross-validation (SECV) and standard errors of prediction (SEP) were both several times larger than the standard errors of calibration (SEC). More recently, Monrroy et al.[9] utilized NIR to estimate the glucan and xylan contents of unbleached kraft *Eucalyptus globulus* pulps.

These previous studies have used different sample preparation methods for collecting NIR spectra. Wallbäcks et al.[7] milled dried pulp samples using a KAMAS Slagy 200 B mill and sieved them through a 1-mm screen, Fardim et al.[8] collected spectra from handsheets prepared with a grammage of 75 g/m^2, while Monrroy et al.[9] examined intact pulp. It is likely that different sample preparation methods could affect the performance of the models, but there have been no studies that compared the effects of different preparation methods applied to the same set of pulp samples. However, there have been many studies that have compared various sample collection and preparation techniques in related areas of NIR research.

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Several studies have examined the performance of wood property calibrations based on milled whole-tree samples when applied to milled increment cores to develop a method for nondestructively sampling living trees.[10–12] Others have investigated whether radial strips cut from increment cores provide better wood property calibrations if the transverse or longitudinal surfaces are exposed to NIR radiation.[13,14] While, wood property calibrations based on green and dry wood have also been compared.[15,16] Despite the diversity of these studies, all used similar statistics to compare the effects of the varying sample collection and preparation techniques on the performance of the corresponding calibrations. Correlation coefficients (R^2), standard errors, and ratios of performance to deviation (RPD) were utilized by the majority of these studies,[11–16] but one study evaluated only correlation coefficients and standard errors.[10]

Although these are excellent statistics for evaluating the performances of individual models, there are problems with using them to assess the relative performances of comparable models, as it is inherently subjective. These statistics give no indication of the probability that the models in question are statistically different from each other, as they do not take sample size into consideration. A more objective approach to evaluating NIR calibrations that are created using different sample preparation would be a significant improvement over previous methods.

Therefore, the objectives of this study are to: (1) identify several possible sample preparation techniques for the NIR analysis of unbleached eucalypt pulps, (2) identify statistical procedures to allow for objective comparisons to be made among the models developed using these different sample preparation techniques, (3) use these statistical procedures to identify the most appropriate sample preparation technique for the NIR analysis of pulp carbohydrate composition, and (4) determine which of the carbohydrates are best-modeled using NIR analysis.

MATERIALS AND METHODS

Sample Origin

Fifty-two 2- to 3-year-old eucalyptus trees were harvested from plantations in two locations of Rio Grande do Sul, Brazil: Colorado (51°51′W and 30°05′S) and Horto Barba Negra (51°14′W and 30°26′S). The trees were chosen to represent a wide range of genetic variability and included six different species and many different hybrids among these species (Table 1). The diameters at breast height (DBH) of the trees ranged from 9 to 18 cm. In addition, seven samples were taken from the factory line of the pulp manufacturing plant in Aracruz, Espírito Santo, Brazil. These were composite samples, as each sample consisted of many different trees, the majority of which were *E. grandis* × *E. urophylla* hybrids. Single-tree samples were used to increase the variability of the dataset without changing the methodology of the pulping process.

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Pulping Process

The single-tree samples were chipped to form whole-tree composites, and 1000 g of the chips were pulped in a laboratory digester to a target Kappa number of 18. The actual Kappa numbers of the single-tree samples ranged from 16.6 to 19.0 (Table 1). The mill composite samples were taken from the Elementary Free Chloride (ECF) factory line, before bleaching took place. The Kappa numbers for the mill composite samples ranged from 15.2 to 16.8.

Pulp Carbohydrate Analysis

The carbohydrate contents of the pulps were determined in acidic hydrozylates using High Performance Liquid Chromatography with Pulsed Amperometric Detection (HPLC–PAD) according to the methodology described by Fardim and Durán [17] The carbohydrates measured were arabinose, fructose, galactose, glucose, rhamnose, and xylose (Table 1).

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Table 1. Statistical summary of carbohydrate content and Kappa numbers of the eucalypt pulps utilized in this study

Species	No. of samples	Statistic	Arabinose (%)	Fructose (%)	Galactose (%)	Glucose (%)	Rhamnose (%)	Xylose (%)	Kappa
E. dunnii	**5**	**Average**	0.0321	0.0078	0.152	76.6	0.0264	18.5	18.1
		Std. Dev.	0.00308	0.00105	0.0360	1.47	0.00167	0.77	0.52
		Minimum	0.0280	0.0064	0.119	75.3	0.0239	17.3	17.2
		Maximum	0.0352	0.0092	0.201	79.1	0.0280	19.4	18.5
E. globulus	**5**	**Average**	0.0389	0.0085	0.257	77.1	0.0239	18.2	17.6
		Std. Dev.	0.00348	0.00191	0.0554	0.56	0.00120	1.21	0.41
		Minimum	0.0370	0.0061	0.183	76.3	0.0225	16.5	17.2
		Maximum	0.0420	0.0110	0.310	77.6	0.0255	19.5	18.0
E. grandis	**5**	**Average**	0.0327	0.0068	0.165	79.0	0.0208	15.4	17.8
		Std. Dev.	0.00337	0.00131	0.0408	0.69	0.00263	1.27	0.45
		Minimum	0.0293	0.0050	0.121	78.1	0.0184	13.4	17.3
		Maximum	0.0372	0.0081	0.206	79.8	0.0239	16.6	18.4
E. maidenii	**5**	**Average**	0.0378	0.0074	0.200	76.9	0.0256	18.7	17.6
		Std. Dev.	0.00353	0.00222	0.0273	1.39	0.00270	1.44	0.67
		Minimum	0.0330	0.0048	0.161	75.3	0.0210	16.3	16.6
		Maximum	0.0414	0.0107	0.227	78.9	0.0282	20.2	18.1
E. saligna	**4**	**Average**	0.0321	0.0066	0.192	79.0	0.0202	16.2	18.1
		Std. Dev.	0.00367	0.00178	0.0234	0.97	0.00145	1.00	0.48
		Minimum	0.0274	0.0046	0.162	77.7	0.1810	14.9	17.5
		Maximum	0.0361	0.0087	0.214	80.0	0.2150	17.4	18.5
E. urophylla	**4**	**Average**	0.0291	0.0088	0.237	78.7	0.0193	16.7	17.9
		Std. Dev.	0.00194	0.00186	0.0270	0.47	0.00153	0.45	0.72
		Minimum	0.0265	0.0065	0.221	78.1	0.0174	16.0	17.5
		Maximum	0.0310	0.0110	0.277	79.2	0.0209	17.1	19.0

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Table 1. Statistical summary of carbohydrate content and Kappa numbers of the eucalypt pulps utilized in this study *(Continued)*

Species	No. of samples	Statistic	Arabinose (%)	Fructose (%)	Galactose (%)	Glucose (%)	Rhamnose (%)	Xylose (%)	Kappa
Hybrids	**24**	**Average**	0.0319	0.0068	0.177	78.0	0.0231	17.1	17.7
		Std. Dev.	0.00429	0.00168	0.0362	1.04	0.00263	1.03	0.42
		Minimum	0.0248	0.0046	0.113	76.0	0.0183	15.2	16.6
		Maximum	0.0398	0.0095	0.258	80.0	0.0273	19.3	18.5
Mill composite samples	**7**	**Average**	0.0274	0.0051	0.165	79.1	0.0162	15.8	16.2
		Std. Dev.	0.00191	0.00152	0.0325	0.69	0.00145	0.30	0.66
		Minimum	0.0247	0.0036	0.133	78.3	0.0140	15.4	15.2
		Maximum	0.0301	0.0071	0.225	80.0	0.0181	16.2	16.8

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Sample Preparation

A handsheet with a grammage of 60 g/m^2 was created for each sample, prepared with a Rapid Köthen apparatus using deionized water. The remainder of each sample was dried as loose pulp. Two disks with a diameter of 38 mm were cut from each handsheet for NIR analysis. These discs are referred to as "handsheets." The loose pulp was divided into three groups. The first group, designated "coarse pulp," was analyzed without any further processing. The second group was sieved through a 7.5 mm screen to separate the finest particles from the coarser material. This material is referred to as "fine pulp." The third group, designated "milled pulp," was milled in a Wiley mill through a 1 mm screen.

NIR Spectroscopy

NIR spectra for each of the four sample preparation methods were measured in a spinning sample holder in a NIRSystems Inc. Model 5000 scanning spectrophotometer. The spectra were collected in diffuse reflectance mode using Vision® software (version 3.1) at 2 nm intervals over the wavelength range 1100–2500 nm. Fifty scans were accumulated for each sample, and the results were averaged. After the spectrum was obtained, the sample cup was emptied, repacked, and a duplicate spectrum was obtained. The data were then imported into the Unscrambler® software (version 9.2) and the duplicate spectra were averaged. The spectra were converted to the second derivative using the Savitsky-Golay convolution algorithm with left and right gaps of 8 nm.[18]

Development of Calibrations and Predictions

The samples were randomly divided into a calibration set approximately two-thirds (40 samples) and a prediction set of one-third (19 samples) (Table 2). Calibrations for each of the four sample preparation methods described were created for each of the six carbohydrates. These calibrations were developed using PLS regression with four cross-validation segments and a maximum of 10 factors.

We observed that the optimum number of factors recommended for each carbohydrate calibration was different as the sample preparation method changed. To facilitate the comparison among calibrations and predictions developed using different preparation methods, the optimum number of factors was held constant by averaging the optimum number of factors determined for each calibration. These calibrations (based on the average number of factors) were then used to predict the values of the samples in their respective prediction sets. The number of factors used for each carbohydrate calibration follow:

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Table 2. Summary statistics for the carbohydrate content and Kappa number of the pulps used in the calibration and prediction sets

	No. of samples	Statistic	Arabinose (%)	Fructose (%)	Galactose (%)	Glucose (%)	Rhamnose (%)	Xylose (%)	Kappa
Calibration	40	Average	0.033	0.0071	0.192	78.2	0.022	17.0	17.6
		Std. Dev.	0.0048	0.00200	0.0411	1.12	0.0035	1.40	0.70
		CoV	0.145	0.282	0.214	0.014	0.159	0.082	0.040
		Minimum	0.025	0.0036	0.121	76.1	0.014	13.4	15.4
		Maximum	0.042	0.0110	0.306	80.0	0.028	19.5	19.0
Prediction	19	Average	0.032	0.0067	0.175	77.7	0.023	17.3	17.7
		Std. Dev.	0.0043	0.00151	0.0518	1.44	0.0039	1.33	0.74
		CoV	0.134	0.225	0.296	0.019	0.170	0.077	0.042
		Minimum	0.025	0.0043	0.113	75.3	0.016	15.2	15.2
		Maximum	0.042	0.0097	0.310	80.0	0.028	20.2	18.4

CoV = coefficient of variation.

arabinose (4), fructose (3), galactose (6), glucose (3), rhamnose (4), and xylose (3).

Data Analyses

Several statistics were used to assess the performance of the calibrations. The standard error of calibration (SEC) (determined from the residuals from the final calibration), standard error of cross-validation (SECV) (determined from the residuals of each cross-validation phase) and the coefficients of determination (R^2) were obtained. The coefficient of determination for calibration (R_c^2) was determined from the residuals of the calibration, while the coefficient of determination for cross-validation ($R^2{}_v$) was determined using residuals of the cross-validation phases. The ratio of performance to deviation (RPD_c), calculated as the ratio of the standard deviation of the reference data to the SECV, was utilized for direct comparisons of calibrations developed for different carbohydrates that have different ranges in values. Similar statistics were used to assess the predictive capability of the calibrations. The standard error of prediction (SEP), the coefficient of determination for prediction (R_p^2) values, and the RPD_p value (ratio of the standard deviation of the reference data to the SEP) were obtained for the prediction sets.

The statistics mentioned above are widely used in the field of NIR spectroscopy to compare calibration performances. However, one goal of this study was to develop a more impartial approach for comparing the predictive capabilities of different calibrations. Thus, we compared the models using a three-way mixed-effects ANOVA using the SAS® (version 9.1) MIXED procedure. The factors included in the analysis were the method of sample preparation, the type of carbohydrate, and sample effect. The sample effect, α_i, was considered a random effect and handles the fact that all of the methods are tested on the same set of samples. The least-squared means for each method were compared using a Tukey test. The following model was used for the analysis:

$$(y - \hat{y})^2 = \mu + \alpha_i + \beta_j + \gamma_k + \beta\gamma_{jk} + \varepsilon_{ijk}$$

where $(y—\hat{y})^2$ = the square of the difference between the measured carbohydrate value and the predicted carbohydrate value

μ = the overall mean
α_i = the effect of sample i
β_j = the effect of method j
γ_k = the effect of carbohydrate k
$\beta\gamma_{jk}$ = the interaction effect of method j and carbohydrate k
ε_{ijk} = the experimental error

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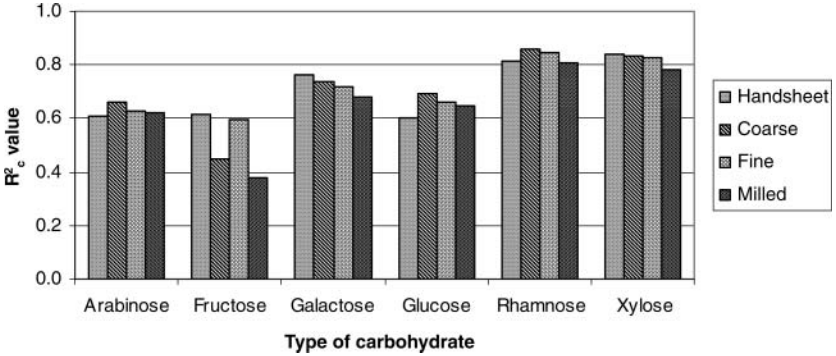

Figure 1. R^2_c values by carbohydrate type, grouped by sample preparation method.

RESULTS

Calibrations statistics varied widely for the six carbohydrates of interest. The calibrations for rhamnose and xylose were the best (Figures 1–3). R^2_c ranged from 0.81 to 0.86 for rhamnose and from 0.78 to 0.84 for xylose (Figure 1). R^2_v were only slightly lower; between 0.77 and 0.82 for both rhamnose and xylose (Figure 2). The RPD_c values were also good for these carbohydrates, but the values for xylose (2.04 to 2.38) were considerably better than those for rhamnose (1.79 to 1.87) (Figure 3). The calibrations for arabinose, fructose, galactose, and glucose were considerably weaker. Although the R^2_c for some of these carbohydrates were reasonable (Figure 1), RPD_c were low, ranging from 0.94 for the coarse pulp fructose calibration to 1.36 for the milled pulp arabinose calibration.

The calibrations were used to predict the carbohydrate contents of the 19 samples in the prediction sets. The prediction statistics were much more

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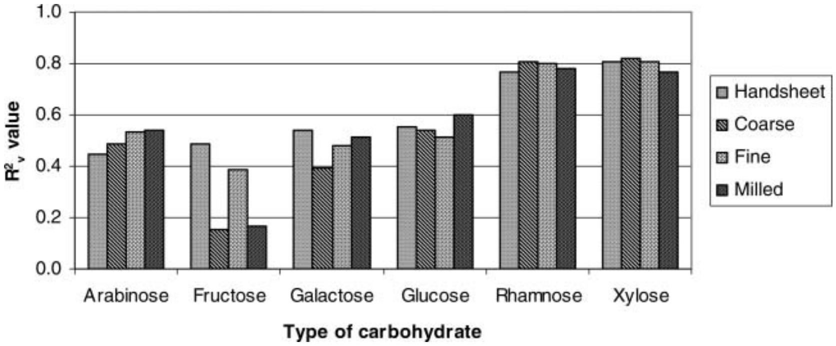

Figure 2. R^2_v values by carbohydrate type, grouped by sample preparation method.

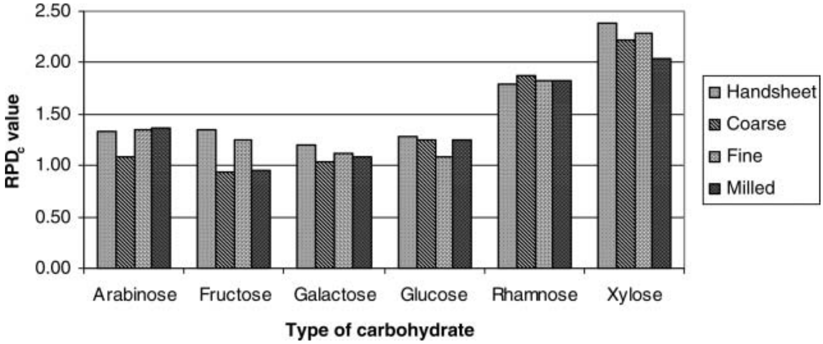

Figure 3. RPD$_c$ values by carbohydrate type, grouped by sample preparation method.

variable than the calibrations statistics. If more samples had been available to test the calibrations this may not have occurred. Again, rhamnose and xylose provided the best statistics; R_p^2 ranged from 0.83 to 0.89 for rhamnose and from 0.79 to 0.82 for xylose (Figure 4). The R_p^2 for the remaining carbohydrates were considerably lower. They were as low as 0.01 to 0.23 for fructose, and as high as 0.50 to 0.60 for glucose (Figure 4).

RPD$_p$ showed significant differences among the four sample preparation methods for each carbohydrate. RPD$_p$ for rhamnose from handsheets was only 1.08, but the values for coarse pulp (2.44), fine pulp (2.41), and milled pulp (2.27) were all considerably higher (Figure 5). For xylose, the values from handsheets (0.98) and milled pulp (0.80) were much lower than the values from coarse pulp (2.01) and fine pulp (1.87). Coarse pulp and fine pulp provided the highest RPD$_p$ for all six carbohydrates (Figure 5).

A three-way random effects ANOVA of the predictions revealed that all of the fixed effects—preparation method, type of carbohydrate, and the

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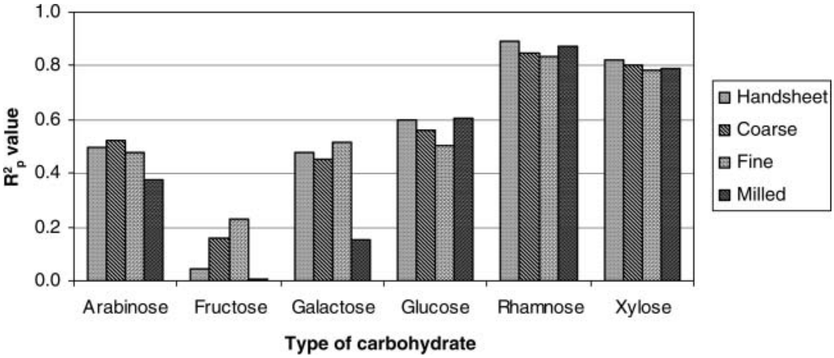

Figure 4. R_p^2 values by carbohydrate type, grouped by sample preparation method.

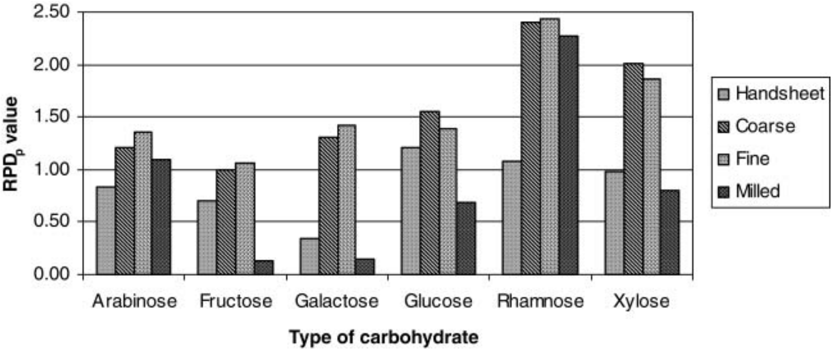

Figure 5. RPD$_p$ values by carbohydrate type, grouped by sample preparation method.

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method*carbohydrate interaction—were statistically significant (Table 3). The significant interaction indicates that the methods performed differently among the carbohydrates. An examination of comparisons revealed several instances where milled pulps performed significantly worse than the other pulp types (for example for glucose: milled vs. coarse, milled vs. fine and milled vs. handsheet and for xylose: milled vs. coarse and milled vs. fine). The explanation for the interaction is that in some cases (those listed here) milled pulp performed significantly worse than the other methods, but in most cases it did not.

The statistical analyses also tested which sample preparation methods resulted in estimates of least mean squares (LMS) that were significantly different from zero. The null hypothesis (H_0) used for this test was LMS $= 0$, while the alternate hypothesis (H_A) was that LMS $\neq 0$. The analysis determined that the estimates for fine pulp, milled pulp, and handsheets were significantly different from zero, but the estimate of LMS for coarse pulp was not significantly different from zero ($\alpha = 0.05$) (Table 4).

Finally, the estimates of LMS for each of the four sample preparation methods were compared, for a total of six comparisons. Differences in the estimates were analyzed with a t-test. No significant differences were found among

Table 3. ANOVA table for the fixed effects of the model. All of the fixed-effect factors were statistically significant

	Numerator degrees of freedom	Denominator degrees of freedom	F-value	p-value
Method	3	54	10.55	<.0001
Carbohydrate	5	360	45.10	<.0001
Method*Carbohydrate	15	360	7.92	<.0001

Table 4. Estimates of the least mean squares (LMS) of the four sample preparation methods. A *t*-test was conducted to test the null hypothesis (H_0) that LMS = 0; the alternate hypothesis (H_A) is that LMS $\neq$ 0. Fine pulp, milled pulp, and handsheets produced least-squares means that were significantly different from zero ($\alpha = 0.05$), but the coarse pulp did not.

Method	Estimate of LMS	*t*-value	*p*-value
Coarse	0.218	1.65	.1044
Fine	0.265	2	.0492
Milled	1.196	9.05	<.0001
Handsheet	0.546	4.13	<.0001

coarse pulp, fine pulp, and handsheets. However, milled pulp was significantly different from each of the methods ($\alpha = 0.05$) (Table 5).

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DISCUSSION

The prediction statistics demonstrate that four of the carbohydrates analyzed in this study (arabinose, fructose, galactose, and glucose) could not be reliably estimated using NIR spectroscopy. However, successful calibrations were created for rhamnose and xylose. These results differ from the findings of Wallbäcks et al.,[7] who found that glucose was very well-modeled by NIR analysis, with xylose and galactose also producing strong correlations. Although Wallbäcks et al.[7] also measured arabinose and mannose, they did not report their findings for these carbohydrates.

Fardim et al.[8] similarly found that xylose and glucose produced good calibrations with NIR analysis. However, their calibrations performed quite poorly in prediction, with the root-mean square error for prediction (RMSEP) for glucose (2.03) nearly 25 times the root-mean square error for calibration

Table 5. Comparison of the estimates of LMS among the four sample preparation methods. There are a total of six comparisons. The *t*-values and *p*-values ($\alpha = 0.05$) are presented for each comparison

Comparison	Difference between LMS's	*t*-value	*p*-value
coarse-fine	–0.047	–0.24	.995
coarse-milled	–0.979	–4.99	<.0001
coarse-handsheet	–0.328	–1.67	.347
fine-milled	–0.931	–4.75	<.0001
fine-handsheet	–0.281	–1.43	.484
milled-handsheet	0.650	3.31	.009

(RMSEC) (0.082). The predictions for xylose were better, but the RMSEP (0.87) was still more than five times the RMSEC (0.165). Although Fardim et al.[8] also measured the mannose, arabinose, galactose, and rhamnose content of the pulps, they did not report NIR analyses of these carbohydrates. In comparison, the recent study by Monrroy et al.[9] reported good calibration statistics for both glucan and xylan.

Based on the previous studies, it was expected that the xylose calibrations would perform reasonably well. However, it was surprising that the glucose calibrations in this study did not perform better, given that glucose accounts for such a large percentage of the total carbohydrates (avg. 78.2%). This is possibly due to the small degree of variability contained in the data set as demonstrated by the very low coefficient of variation (0.014) compared to the other sugars (Table 2). The excellent calibration and prediction statistics for rhamnose were unexpected. Rhamnose composed, on average, only 0.022% of the total mass of the pulps in this study. While its coefficient of variation (0.16) was much higher than that of glucose it was actually less variable than either fructose or galactose (Table 2), two carbohydrates whose calibrations were much poorer.

Of the four sample preparation methods that were compared in this study, only Monrroy et al.[9] examined intact pulps (Wallbäcks et al.[7] used milled pulp, while Fardim et al.[8] used handsheets to collect spectra). It was unexpected that the intact pulps consistently resulted in the best calibration statistics. Although a statistical significance test did not find these methods to be significantly better than handsheets (Table 5), this may be due to the small size of our prediction set (19 samples). Milled pulp, which provided good results for previous researchers[7] performed significantly worse than both coarse and fine pulp. This result is quite important, given the additional time and equipment required to mill pulp into a fine powder. It is possible that because the small size of the sieve openings (1 mm) allowed only the finest particles to pass through, the portions that we collected the spectra from were not representative of the entire samples and led to poor estimates. No differences were found among models for handsheets and coarse and fine pulps, as coarse pulp requires the least preparation, we recommend that this sample preparation technique be used for pulp carbohydrate analysis by NIR spectroscopy.

CONCLUSIONS

Four different sample preparation methods (coarse, fine, and milled pulp, and handsheets) were used to analyze the carbohydrate content of unbleached eucalyptus pulps (52 single-tree, 7 mill composite) with NIR spectroscopy. Models with good predictive capability were obtained for rhamnose and xylose, but models for arabinose, fructose, galactose, and glucose did not perform as well. The models, based on NIR spectra obtained from samples prepared using the 4 different methods, were compared using a three-way mixed-effects ANOVA.

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Coarse and fine pulp preparation methods consistently yielded the best calibration and prediction statistics, although they were not significantly better than prediction statistics for handsheets. Milled pulp produced the worst prediction statistics. Given its ease of preparation, coarse pulp is the recommended method for collecting spectra for carbohydrate content analysis by NIR spectroscopy.

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