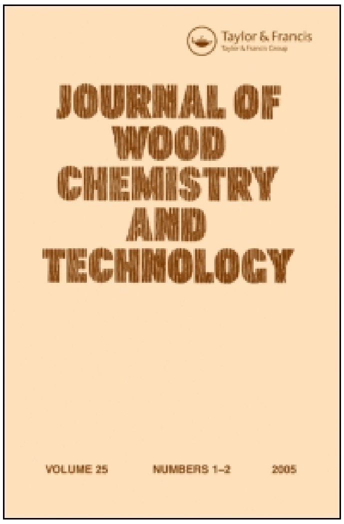

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T. Ona[a]; T. Sonoda[a]; K. Ito[a]; M. Shibata[a]; T. Kato[b]; Y. Ootake[b]
[a] Kameyama Research Center, Forestry Research Institute, Kameyama, Japan [b] Aichi-ken Agricultural Research Centre, Nagakute, Japan

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NON-DESTRUCTIVE DETERMINATION OF WOOD CONSTITUENTS BY FOURIER TRANSFORM RAMAN SPECTROSCOPY

T. Ona[1], T. Sonoda[1], K. Ito[1], M. Shibata[1], T. Kato[2] and Y. Ootake[2]
[1]Kameyama Research Center, Forestry Research Institute, Oji Paper,
24-9 Nobono-cho, Kameyama 519-02, Japan
[2]Aichi-ken Agricultural Research Centre,
1-1 Sagamine, Yazako, Nagakute 480-11, Japan

ABSTRACT

The feasibility of using FT-Raman spectroscopy for rapid determination of various wood constituents non-destructively was examined using five *Eucalyptus* species, including samples of various ages and colors of samples, which are of importance as a plantation source. Wood constituents which relate to pulp properties (holocellulose, α -cellulose, hemicellulose, lignin, extractives, alkali-extractives, total-extractives, and extractives-free (EF) wood constituents for holocellulose, α -cellulose, hemicellulose and lignin) were measured. The application of 2nd derivatives transformation of Raman spectroscopic data revealed highly significant correlations between wet chemical and Raman predicted values for all traits except EF-hemicellulose, with standard error of prediction (SEP) < 0.8 points in the calibration (for known samples) and SEP < 3.4 points in the prediction (for unknown samples), respectively. Consequently, this non-destructive method has proved its validity for analyzing various *Eucalyptus* native wood meal samples, regardless of their age and color, to determine wood constituents and EF-wood constituents except hemicellulose. Use of this method will reduce the costs of tree improvement programs and the minimal sample size needed will allow trees to be non-destructively sampled.

INTRODUCTION

Forest resources, particularly plantation trees have become increasingly important as renewable sources of raw materials during the last few decades. Advantage can be taken of breeding programs to achieve the improvements in tree growth rate and quality and to contribute to environmental issues by reducing reliance on natural forests. Furthermore, plantation trees can reduce the manufacturing costs of pulp and paper products effectively.

Tree selection is preferably performed by use of an increment core method, which retains the tree for future breeding and propagation. To achieve this, we have developed the Radially divided Increment Core (RICO) method and determined the appropriate sampling position to assess whole-tree pulp properties by chemical analysis of the wood properties of a single core.[1] The advantage of this method is that we can estimate many pulp properties under various cooking conditions by using the relationship between pulp and wood properties obtained under each cooking condition. However, even if we use the 'Small-scale method' which can handle 200 mg each of 12 wood samples at once,[2] determination of wood properties for hundreds of trees is still time consuming.

Several instrumental techniques such as ^{13}C-nuclear magnetic resonance (NMR) spectroscopy, fourier transform infrared (FTIR) spectroscopy and near-infrared (NIR) spectroscopy, have been developed in recent years. These have been applied to determine wood constituents.[3-7] However, NMR has the disadvantage of long analytical time and FTIR quite often requires hazardous sample preparation wherein the samples have to be mixed with potassium bromide. In contrast to these, NIR is a rapid method with easy sample preparation as wood powder can be analyzed directly.[4,7] NIR has been successfully utilized to determine cellulose content,[4] lignin content,[7] hot water solubles and alkali solubles contents.[7] However, the estimate of various pulp properties by wood properties requires more traits than described above.[1]

On the other hand, Fourier transform Raman (FT-Raman) spectroscopy has only very recently been applied to examine wood constituents.[8-10] The advantages of FT-Raman over FTIR and NIR are as follows; (1) water is not a disturbance,[11] (2) ease of analyzing with heterogeneous samples,[11] and (3) ease of analyzing structural features of samples.[10, 12] However, little has been reported on the application of FT-Raman to determine, in a non-destructive manner, the content of wood constituents.

Here, we report on the feasibility of using FT-Raman as a rapid non-destructive means of determining the content of various wood constituents using five *Eucalyptus* species which are of importance as a plantation source. We also report on the successful application of the 2nd derivatives transformation of Raman spectroscopic data to recognize important frequencies for wood constituent determination.

RESULTS AND DISCUSSION

Wet chemical data of all traits are summarized in Table 1 and are not significantly different between in calibration and in prediction.

Figure 1A shows an example of the Raman spectra and Figure 1B its 2nd derivatives form. The 2nd derivatives form gives us more information on important frequencies which are hidden by overlapped signals in the Raman spectra, e.g. at the frequency 1595 cm^{-1}. Consequently, it was the 2nd derivatives form that was used in this study.

Plots of the calibration by the partial least squares (PLS) regression are shown in Figures 2 and 3, and the statistical results are summarized in Table 2.

PLS regression is one of the processes of multivariate data analysis which models the relationship between two data sets,[13] and is a bilinear modeling method.[14] In this method, the information in the original X-data (as the intensities at different

TABLE 1
Wet Chemical Data of Contents of Wood Constituents

Trait	% in Calibration (n=63)	% in Prediction (n=30)
Holocellulose	79.9 ± 6.8	79.6 ± 7.4
α -Cellulose	44.9 ± 6.6	44.0 ± 5.8
Hemicellulose	35.0 ± 2.9	35.6 ± 3.7
Lignin	18.8 ± 3.5	18.7 ± 2.8
Extractives	6.5 ± 4.6	7.6 ± 5.3
Alkali-extractives	12.2 ± 3.2	12.0 ± 3.0
Total-extractives	18.7 ± 4.8	19.7 ± 4.4
EF-holocellulose	85.4 ± 4.4	86.0 ± 4.0
EF- α -cellulose	47.9 ± 5.7	47.5 ± 4.6
EF-hemicellulose	39.2 ± 4.2	39.3 ± 3.9
EF-lignin	20.2 ± 4.0	20.4 ± 3.7

EF; extractives-free

frequencies of Raman shift in the present study) is first projected onto a small number of underlying variables called principal components. Secondary, the Y-data (as wood constituents determined by wet chemical method in the present study) are actively used in estimating the principal components to confirm that the first components are most relevant for predicting the Y-data. Thirdly, the interpretation of the relationship between two data sets is then concentrated on the smallest possible number of components.

In the PLS regression, the number of principal components used in the model for each wood constituent was decided by the residual variance which was defined as the mean squared residual corrected for degrees of freedom.

As shown in Figures 2 and 3, and Table 2, we successfully obtained highly significant correlation coefficients over 0.99 between wet chemical and Raman predicted values for all traits. This was the first successful calibration of the contents of wood constituents using native wood meals and FT-Raman. Furthermore, since all traits except cellulose and lignin have not been calibrated even by NIR,[4, 7] the results obtained are remarkable. Considering wet chemical values have less than 2 points standard deviation by a small-scale method,[2] the achieved level of calibration

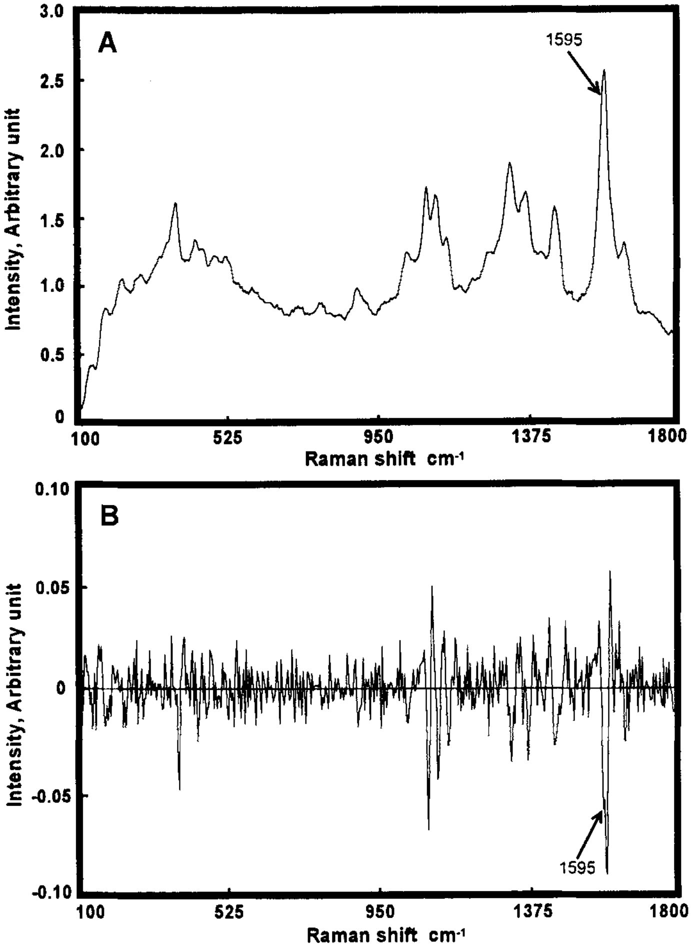

FIGURE 1. Normal FT-Raman spectra (A) and its 2nd derivatives form (B) of a *E. camaldulensis* sample.

for each trait will be highly significant because the standard error of prediction (SEP) was less than 0.8 points.

In addition to this, the samples utilized in the calibration have important implications as each calibration model was created using four different *Eucalyptus*

TABLE 2
Statistical Result in Calibration

Trait	r	SEP	No. of PCs used
Holocellulose	0.994	0.760	5
α-Cellulose	0.993	0.762	5
Hemicellulose	0.994	0.309	6
Lignin	0.998	0.239	6
Extractives	0.994	0.495	5
Alkali-extractives	0.994	0.334	6
Total-extractives	0.997	0.344	6
EF-holocellulose	0.996	0.384	6
EF-α-cellulose	0.993	0.695	5
EF-hemicellulose	0.994	0.462	6
EF-lignin	0.994	0.448	5

EF; extractives-free, r; correlation coefficients, SEP; standard error of prediction, PC; principal component

species, including samples taken of different ages from various parts within tree stems, and a sample of *E. grandis* chips of unknown age. Moreover, it should be difficult to obtain calibrations using different species in FT-Raman because of the base line change in the spectra, due to the difference in florescence intensity of colored materials.[9] In fact, some parts of the *E. camaldulensis* No. 1 and 2, and *E. Trabutii* samples were colored red due to the heartwood formation.[15] Consequently, each calibration will be valid for various *Eucalyptus* native wood meal samples, regardless of their age and heartwood color. These results are unique among the few reports on hardwood FT-Raman and NIR.

Tables 3-5 show important frequencies selected by regression coefficients used in the calibration model for traits, namely holocellulose, α-cellulose, hemicellulose, lignin, extractives and alkali-extractives. Plus and minus followed by frequencies express positive and negative regression coefficients, respectively. Traits not listed in Tables 3-5 were omitted because they are calculated values of listed traits. In Tables 3-5, important frequencies in the calibration models were assigned according to the references and Raman spectra of untreated wood, extractives-free (EF) wood,

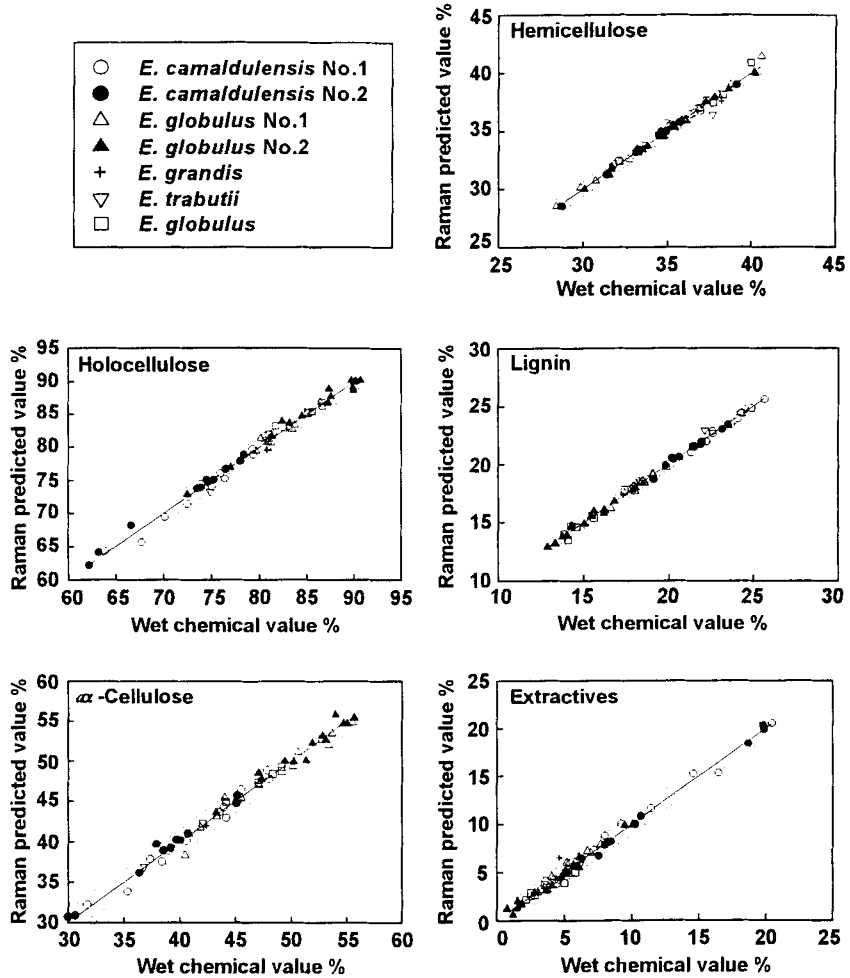

FIGURE 2. Plots of FT-Raman calibration (for known samples) against the contents of wood constituents Part 1. The 95% confidence contours are represented by dashed lines.

holocellulose and α -cellulose in Figure 4 although the assignment is only tentative. Table 6 shows correlation between the traits.

The correlations between traits in Table 6 are in good agreement with many of the assigned frequencies for the traits in Tables 3-5. Some frequency assignments

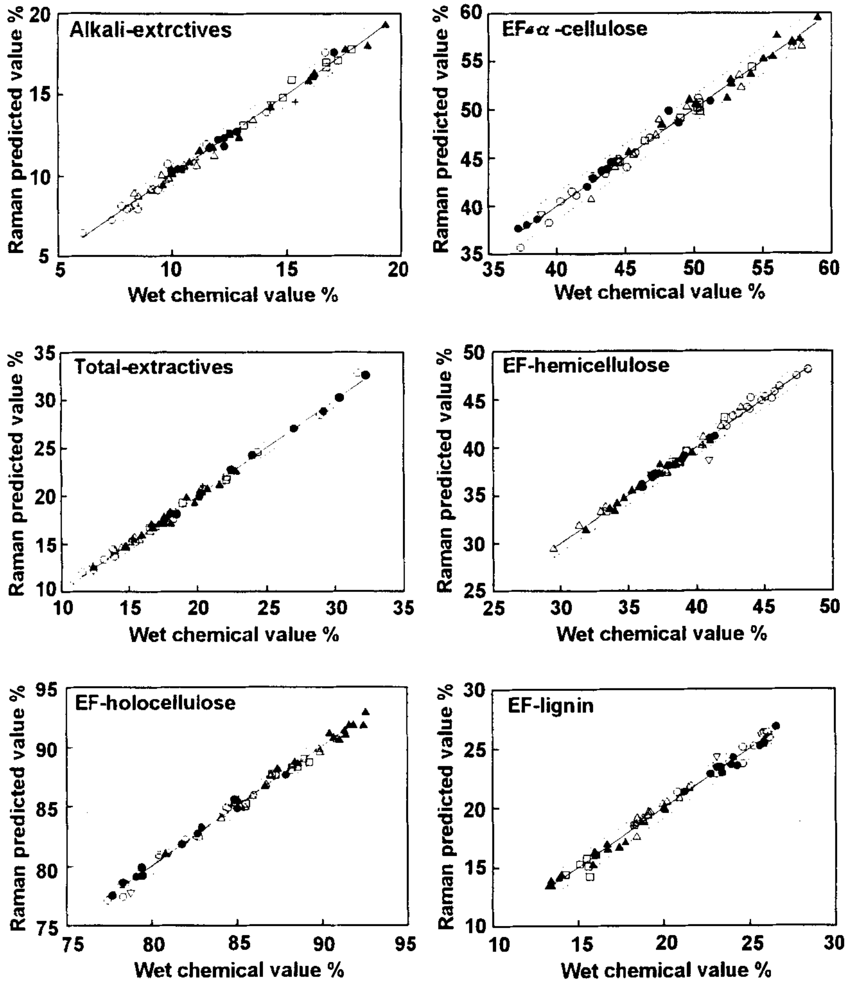

FIGURE 3. Plots of FT-Raman calibration (for known samples) against the contents of wood constituents Part 2. See legends in Figure 2. The 95% confidence contours are represented by dashed lines.

were derived from the correlations between traits, including extractives in holocellulose trait and lignin in the alkali-extractives trait. Frequencies less than 200 cm^{-1} were included and have been assigned based on the spectral changes during the chemical treatment of samples (Figure 4). The results suggest that, although the

TABLE 3
Important Frequencies in the Calibration Models with Possible Assignments for Holocellulose and α-Cellulose

Trait	Raman Shift cm^{-1}	Assignment	Ref.
Holocellulose	+ 781	• See Extractives	
	+ 1618	• See Extractives	
	− 1641	• Ketone	6
		• Carbonyl-bearing moieties by the reaction between Cl_2 and lignin	9
		• C=O stretch	7
	+ 1622	• See Extractives	
	− 1583	• See Hemicellulose	
	− 345	• See α-Cellulose	
	+ 1614	• See Extractives	
	− 183	• See α-Cellulose	
	+ 1610	• See Extractives	
	− 114	• Extractives	–
α-Cellulose	+ 781	• See Extractives	
	− 376	• Cellulose	11
	− 345	• Cellulose	11
	+ 341	• Cellulose	–
	+ 168	• Hemicellulose	–
	− 1641	• See Holocellulose	
	+ 1618	• See Extractives	
	− 183	• Hemicellulose	–
	+ 1610	• See Extractives	
	+ 1714	• Extractives + lignin + hemicellulose	–
		• Benzene substitutes	8
		• Carbonyl group	8

Plus and minus followed by frequencies express positive and negative regression coefficients, respectively.

Raman intensity is rather low, it is possible to utilize short frequencies in the chemical study of wood.

The holocellulose frequency 1641 cm^{-1} was previously assigned as carbonyl bearing moieties in lignin by the reaction between chlorine and lignin based on the spectral changes between untreated wood and holocellulose.[9] In this study, its intensity was enhanced in holocellulose samples. Consequently, the frequency 1641

TABLE 4

Important Frequencies in the Calibration Models with Possible Assignments for Hemicellulose and Lignin

Trait	Raman Shift cm^{-1}	Assignment	Ref.
Hemicellulose	+ 1618	• See Extractives	
	− 1637	• Extractives + lignin + hemicellulose	–
		• Benzene substitutes	8
		• Carbonyl group	8
	+ 1622	• See Extractives	
	− 1464	• See Lignin	
	+ 1614	• See Extractives	
	+ 1452	• Extractives + lignin	–
		• Benzene substitutes	8
		• Carbonyl group	8
	+ 588	• Extractives + lignin	–
	− 1329	• Hemicellulose	–
		• Carbonyl group	8
	+ 1479	• Extractives	–
		• Benzene substitutes	8
		• Carbonyl group	8
	− 1583	• Hemicellulose	–
		• Carbonyl group	8
Lignin	− 1595	• Lignin	9
	− 584	• Extractives + lignin	–
	− 1398	• Extractives + lignin	–
		• Aromatic aldehyde	8
	− 1603	• Lignin	11
	+ 1379	• Cellulose	11
	+ 928	• Lignin	11
	+ 580	• Extractives + Lignin	–
	− 1510	• Lignin	9
	+ 1464	• Lignin	10
	+ 1093	• Lignin	11

Plus and minus followed by frequencies express positive and negative regression coefficients, respectively.

cm^{-1} is considered to have originated from hemicellulose, especially the carbonyl bonds[17] in acetyl group of xylan and arabinogalactan (galactan).

Many of the frequencies for the traits extractives and alkali-extractives are derived from benzene substitutes and carbonyl groups. These correspond to the

TABLE 5
Important Frequencies in the Calibration Models with Possible Assignments for Extractives and Alkali-extractives

Trait	Raman Shift cm^{-1}	Assignment	Ref.
Extractives	– 781	• Extractives	–
		• Benzene substitutes	8
	– 1618	• Hot water soluble	7
		• C=C stretch in coniferaldehyde	8
	– 1614	• Benzene ring stretch	9
		• Carbonyl group	9
	+ 1641	• See Holocellulose	
	– 777	• Extractives	–
		• Benzene substitutes	8
	– 1622	• Hot water soluble	7
		• C=C stretch in coniferaldehyde	8
	– 1610	• Benzene ring stretch	9
		• Carbonyl group	9
	+ 1637	• See Hemicellulose	
	+ 758	• Extractives	–
		• Benzene substitutes	8
Alkali-extractives	– 1765	• Extractives	–
		• Benzene substitutes	8
	– 1579	• Extractives	–
		• Benzene substitutes	8
	+ 974	• Cellulose	11
	+ 1792	• Extractives	–
		• Benzene substitutes	8
	– 106	• Extractives	–
	+ 1610	• See Extractives	
	– 982	• Extractives + hemicellulose	–
	– 1417	• Aromatic aldehyde	9
	– 1468	• Lignin	11
	– 1780	• Lignin	11

Plus and minus followed by frequencies express positive and negative regression coefficients, respectively.

extraneous compounds in *Eucalyptus* species, such as polymerized anthocyanidins, elagic and gallic acids,[19, 20] with further assignments expected.

Figures 5 and 6, and Table 7 show the prediction of each trait by Raman spectroscopy using the obtained calibration model.

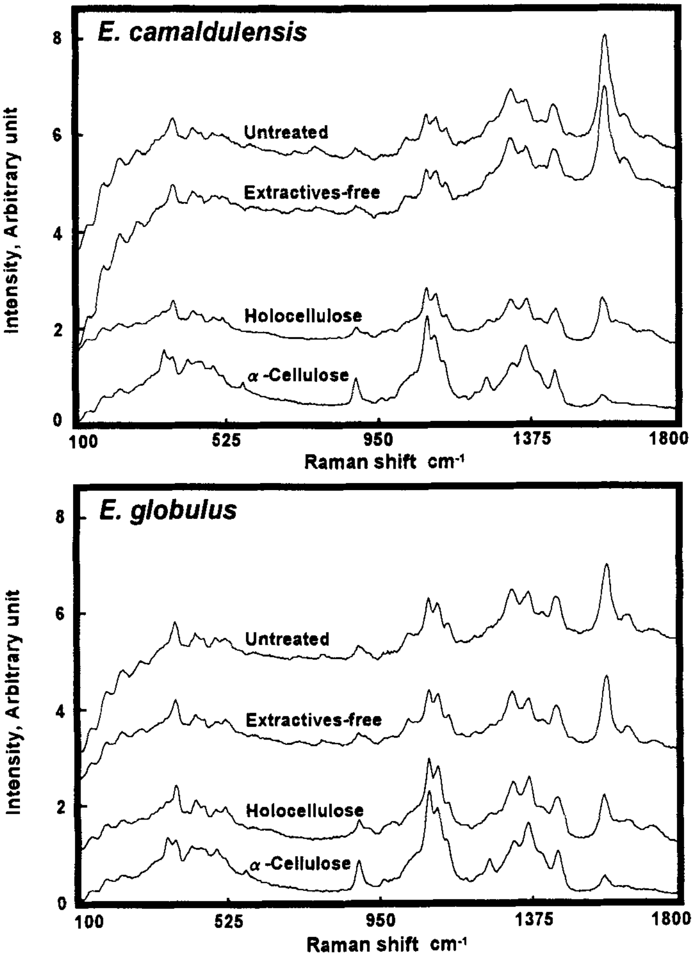

FIGURE 4. FT-Raman spectra of untreated and various treated samples in *E. camaldulensis* and *E. globulus*.

TABLE 6

Correlation Coefficients between Traits

	Holo-cellu-lose	α-Cellu-lose	Hemi-cellu-lose	Lignin	Extrac-tives	Alkali-extrac-tives	Total-extrac-tives	EF-holo-cellu-lose	EF-α-cellu-lose	EF-hemi-cellu-lose	EF-lignin
Holo-cellulose	—	*** 0.909	** 0.274	*** -0.571	*** -0.837	** 0.286	*** -0.614	*** 0.864	*** 0.800	** -0.326	*** -0.747
α-Cellulose		—	-0.152	*** -0.531	*** -0.708	0.140	*** -0.585	*** 0.835	*** 0.962	*** -0.558	*** -0.676
Hemi-cellulose			—	-0.128	*** -0.351	*** 0.354	-0.105	0.120	*** -0.323	*** 0.512	* -0.212
Lignin				—	* 0.211	*** -0.503	-0.129	*** -0.728	*** -0.563	*** 0.570	*** 0.962
Extractives					—	** -0.275	*** 0.777	*** -0.450	*** -0.493	0.072	*** 0.468
Alkali-extractives						—	*** 0.391	0.199	0.057	-0.192	*** -0.525
Total-extractives							—	** -0.299	*** -0.434	-0.057	0.103
EF-holo-cellulose								—	*** 0.858	*** -0.464	*** -0.782
EF-α-cellulose									—	*** -0.658	*** -0.645
EF-hemi-cellulose										—	*** 0.536
EF-lignin											—

* * * Significant at 1% level of analysis of variance (ANOVA), * * Significant at 5% level of ANOVA, * Significant at 10% level of ANOVA, EF; extractives-free

For all traits, except EF-hemicellulose, highly significant correlations were obtained between wet chemical and Raman predicted values. The SEPs were less than 3.4 points using samples of different species, age and color, that included different positions within the stems of *E. camaldulensis* and *E. globulus*, and also samples of *E. nitens* which was not included in the calibration. The non-destructive FT-Raman spectroscopic method has proved its validity for determining the content of wood constituents for *Eucalyptus* native wood meal samples, regardless of their age and color. The method also allows us to determine the content of extractives-free wood constituents except hemicellulose. The results are considered unique.

Use of this method will reduce the costs of tree improvement programs and the minimal sample size needed will allow trees to be non-destructively sampled.

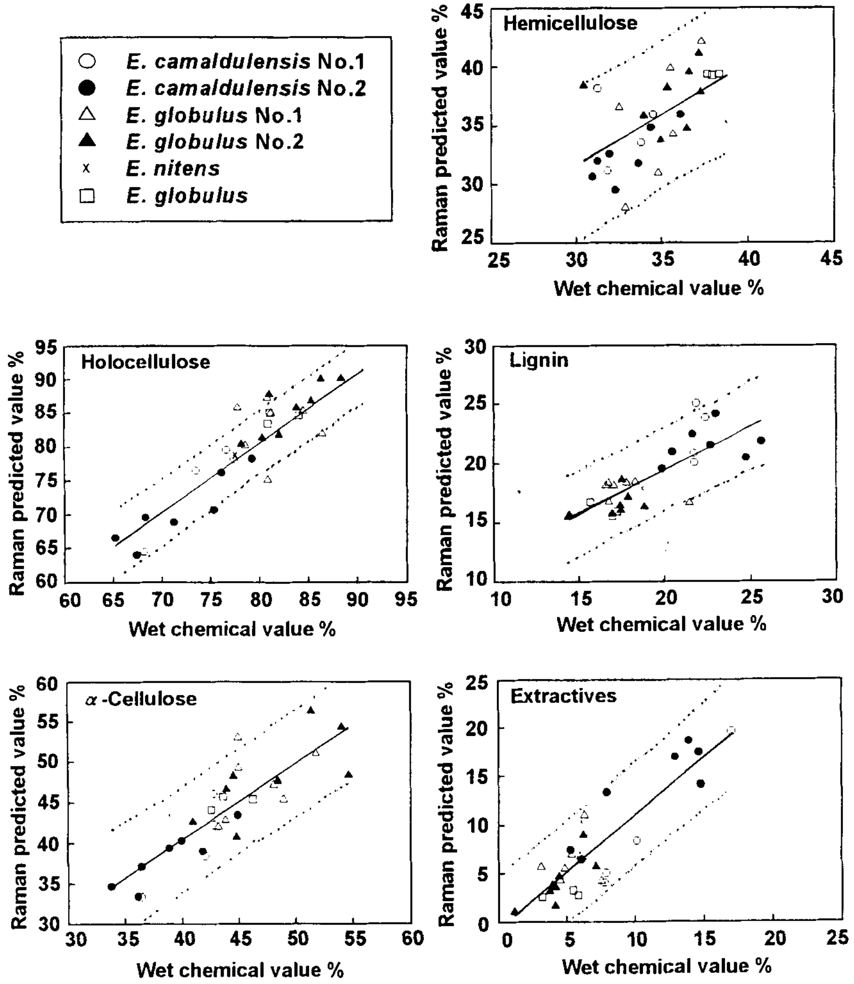

FIGURE 5. Plots of FT-Raman prediction (for unknown samples) against the contents of wood constituents Part 1. The 95% confidence contours are represented by dashed lines.

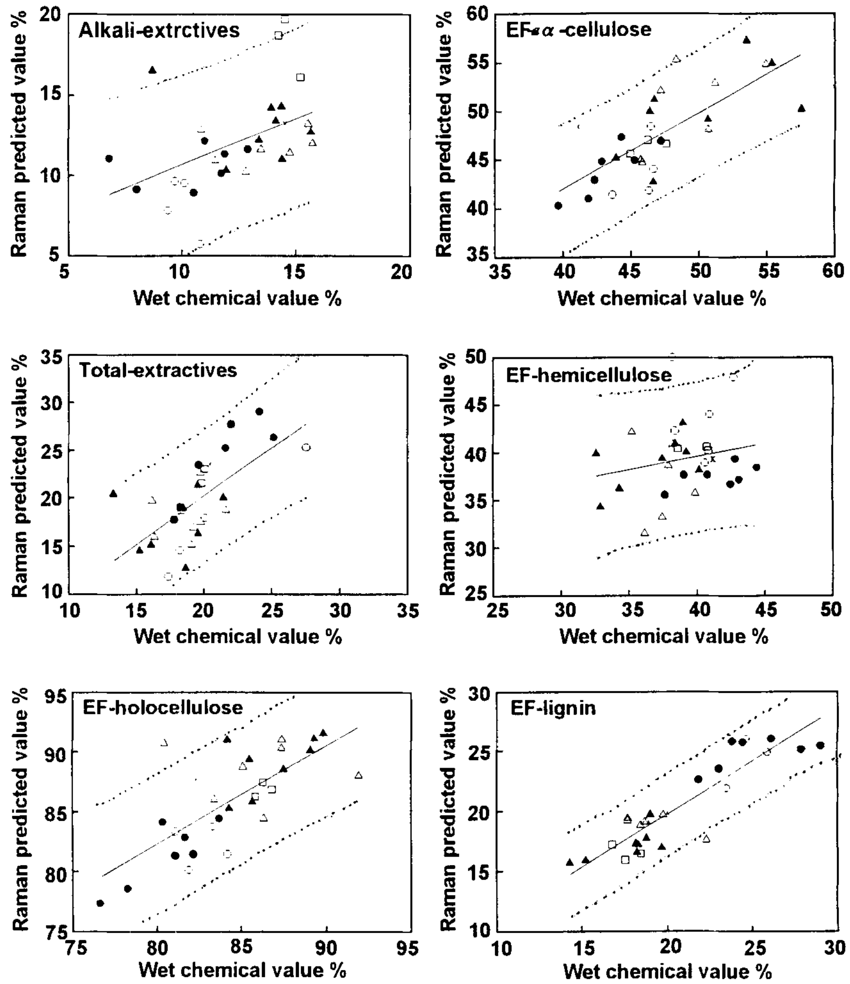

FIGURE 6. Plots of FT-Raman prediction (for unknown samples) against the contents of wood constituents Part 2. See legends in Figure 5. The 95% confidence contours are represented by dashed lines.

TABLE 7
Statistical Result in Prediction

Trait	r	SEP
Holocellulose	0.902	3.296
α-Cellulose	0.843	3.131
Hemicellulose	0.570	3.033
Lignin	0.792	1.850
Extractives	0.880	2.596
Alkali-extractives	0.469	2.834
Total-extractives	0.662	3.314
EF-holocellulose	0.724	2.811
EF-α-cellulose	0.732	3.245
EF-hemicellulose	0.209	4.314
EF-lignin	0.893	1.719

EF; extractives-free, r; correlation coefficients, SEP; standard error of prediction

EXPERIMENTAL

Materials

Eucalyptus nitens (2 years old) and *E. trabutii* (2.5 years old) samples were grown at Oji Paper (Kameyama, Japan). The *E. grandis* sample was imported from the Republic of South Africa as chips. The *E. camaldulensis* samples (two 14 year-old trees, numbered 1 and 2), and *E. globulus* samples (two 14 year-old, numbered 1 and 2; ten 9.5 year-old trees) were grown at CALM (Department of Conservation and Land Management, Western Australia, Australia). The *E. camaldulensis* samples included 19 block samples, each about 2 x 2 x 6 cm, from trees No. 1, 18 block samples from tree No. 2. The *E. globulus* included 20 block samples from No.1, and 23 block samples from tree No.2. The block samples were taken randomly in the stems, and had been utilized in an earlier study.[1]

Wet chemical method

The contents of wood constituents were determined by a small-scale method[2] for holocellulose, α-cellulose, hemicellulose, lignin, and extractives (extraneous compounds dissolved in a Soxhlet apparatus using a sequence of toluene/ethanol, ethanol and water), alkali-extractives and total-extractives (extractives plus alkali-extractives) as extraneous compounds. The contents of EF-wood constituents were calculated as (contents of wood constituents by original wood basis) x (100 - extractives content %) / 100 for holocellulose, α-cellulose, hemicellulose and lignin.[1] The correlations between wood constituents were calculated by ANOVA with the software of SPSS (SPSS Inc., IL, USA).

Raman spectroscopy

Raman spectra were collected using a Nicolet Raman 950 spectrometer (Nicolet Instrument Corp., Madison, USA) equipped with a Nd: YAG laser operating at 1064 nm, and Ge detector. Wood meal samples (20 mesh pass) were packed into NMR tubes and spectra were measured using 180° backscattering at 500 mW, 4 cm^{-1} resolution and 256 scans. Raman spectra of 100-1800 cm^{-1} frequencies were transformed to the 2nd derivatives form after the multiplicative scatter correction. They were then subjected to the PLS regression with the contents of wood constituents by using the software of Unscrambler 6.0 (Camo AS, Trondheim, Norway). A total of 63 samples for calibration and 30 for prediction were selected randomly.

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REFERENCES

1. T. Ona, T. Sonoda, K. Ito, M. Shibata, Y. Tamai and Y. Kojima, Appita, 49, 325 (1996).
2. T. Ona, T. Sonoda, M. Shibata and K. Fukazawa, Tappi, 78, 121 (1995).
3. S. A. Berben, J. P. Rademacher, L. O. Sell and D. B. Easty, Tappi, 70, 129 (1987).
4. J. A. Wright, M. D. Birkett and M. J. T. Gambino, Tappi, 73, 164 (1990).
5. L. Wallbäcks, U. Edlund, B. Nordén and I. Berglund, Tappi, 74, 201 (1991).
6. S. Backa and A. Brolin, Tappi, 74, 218 (1991).
7. A. J. Michell, Appita, 47, 29 (1994).
8. U. P. Agarwal, R. H. Atalla and I. Forsskåhl, Holzforschung, 49, 300 (1995).
9. D. Stewart, H. M. Wilson, P. J. Hendra and I. M. Morrison, J. Agric. Food Chem., 43, 2219 (1995).
10. T. Takei, N. Kato, T. Iijima and M. Higaki, Mokuzai Gakkaishi, 41, 229 (1995).
11. U. P. Agarwal and R. H. Atalla, In Surface Analysis of Paper, T. E. Conners and S. Banerjee (eds.), p. 152-181, CRC Press, FL, 1995.
12. V. M. Hallmark, In Fourier Transform Raman Spectroscopy: From Concept to Experiment, D. B. Chase and J. F. Rabolt (eds.), p. 73-110, Academic Press, San Diego, New York, Boston, London, Sydney, Tokyo, Toronto, 1994.
13. K. R. Beebe and B. R. Kowalski, Anal. Chem., 59, 1007 (1987).
14. Anon, The Unscrambler 6.0 User's Guide, p. 298, Camo AS, Tronheim, Norway, 1996.
15. T. Ona, T. Sonoda, K. Ito and M. Shibata, Jpn. Tappi, 49, 861 (1995).
16. W. Kemp, Organic Spectroscopy, p. 42-52, MacMillan Education, London, 1991.

17. F. S. Parker, Applications of Infrared, Raman, and Resonance Raman Spectroscopy in Biochemistry, p. 27, Plenum Press, New York, London, 1983.

18. R. M. Silverstein, G. C. Bassler and T. C. Morrill, Spectrometric Identification of Organic Compounds, 5th ed. (Japanese Ed.), p. 83-152, John Wily & Sons (Tokyo Kagaku Dojin in Japan), 1991 (1992).

19. W. E. Hillis, In Eucalypts for wood production, W. E. Hillis and A. G. Brown (eds.), p. 259-288, CSIRO, Australia, 1978.

20. H. G. Higgins, In Eucalypts for wood production, W. E. Hillis and A. G. Brown (eds.), p. 290-316, CSIRO, Australia, 1978.