

FTIR Spectroscopic Prediction of Klason and Acid Soluble Lignin Variation in Norway Spruce Cutting Clones

Sanni Raiskila, Minna Pulkkinen, Tapio Laakso, Kurt Fagerstedt, Mia Löija, Riitta Mahlberg, Leena Paajanen, Anne-Christine Ritschkoff and Pekka Saranpää

Raiskila, S., Pulkkinen, M., Laakso, T., Fagerstedt, K., Löija, M., Mahlberg, R., Paajanen, L., Ritschkoff, A.-C. & Saranpää, P. 2007. FTIR spectroscopic prediction of Klason and acid soluble lignin variation in Norway spruce cutting clones. *Silva Fennica* 41(2): 351–371.

Our purpose was to develop a FTIR spectroscopic method to be used to determine the lignin content in a large number of samples and to apply this method studying variation in sapwood and heartwood lignin content between three fast-growing cutting clones grown in three sites. Models were estimated with 18 samples and tested with 6 samples for which the Klason lignin + acid soluble lignin content had been determined. Altogether 272 candidate models were built with all-subset regressions from the principal components estimated from differently treated transmission spectra of the samples; the spectra were recorded on KBr pellets of sieved and unsieved unextracted wood powder and subjected to four different preprocessings and two different wavenumber selection schemes. The final model showed an adequate fit in the estimation data ($R^2=0.74$) as well as a good prediction performance in the test data ($R^2_p=0.90$). This model was based on the wavenumber range of $1850\text{--}500\text{ cm}^{-1}$ of the line-subtraction-normalised spectra recorded from sieved samples. The model was used to predict lignin content in 64 samples of the same material. One of the clones had a slightly lower sapwood lignin content than the two other clones. The fertile growing site with fast growing trees showed slightly higher sapwood lignin content compared with the other two sites. The model was also used to predict the lignin content in the earlywood of 45 individual annual rings. Variation between individual stems and between annual rings was found to be large. No correlation was found between the lignin content and density of earlywood.

Keywords FTIR, lignin, Norway spruce, PCR, principal component regression

Authors' addresses *Saranpää, Raiskila and Laakso*, Finnish Forest Research Institute, Vantaa Research Unit, P.O. Box 18, FI-01301 Vantaa, Finland; *Pulkkinen*, Department of Forest Ecology, P.O. Box 27, FI-00014 University of Helsinki, Finland; *Fagerstedt*, Department of Biological and Environmental Sciences, Plant Biology, P.O. Box 65, FI-00014 University of Helsinki, Finland; *Ritschkoff, Löija, Mahlberg and Paajanen*, VTT Building and Transport, P.O. Box 1806, FI-02044 VTT, Finland **E-mail** pekka.saranpaa@metla.fi

Received 26 July 2006 **Revised** 22 January 2007 **Accepted** 13 March 2007

Available at <http://www.metla.fi/silvafennica/full/sf41/sf412351.pdf>

1 Introduction

The conifer cell wall consists of 40–50% cellulose, 20–35% hemicellulose and 15–35% lignin (Panshin and deZeeuw 1980, Sjöström 1993, Walker 1993). In the stem wood of Norway spruce the mean lignin content (gravimetric lignin + acid soluble lignin) has been reported to be 28.9% with standard deviation of 0.8% (Anttonen et al. 2002, Anttonen, personal communication). The variation of lignin content in wood raw material causes problems to wood-utilizing industry, which endeavours to assure a uniform quality of the end products. On the other hand, the natural genetic variation in trees provides an opportunity to select individuals with desirable lignin contents for breeding. So far, however, the genetic variation in lignin content or composition in trees has been studied very little.

The lignin content has traditionally been determined by wet chemical methods e.g. with the acetyl bromide method (spectrophotometric method) suitable for a small amount of sample (Iiyama and Wallis 1988, Hatfield et al. 1999, Fukushima and Hatfield 2001, Hatfield and Fukushima 2005) and with the Klason lignin (gravimetric method) and acid soluble lignin (spectrophotometric method) measurement (Dence 1992) suitable for a large amount of sample. In the first method the ground extracted wood is dissolved in acetyl bromide in acetic acid containing perchloric acid and in the latter methods degraded with sulphuric acid (Iiyama and Wallis 1988). The extraction is often, e.g. with the Soxhlet method, slow and consumes a large amount of organic solvent. Hence, it is unfeasible for large sets of samples. A rapid and reproducible method for screening of lignin content, as well as other cell wall properties, would thus be welcome to practical wood use and tree breeding purposes.

Transmission or diffuse reflectance spectra in the mid infrared or near infrared regions (NIR) are fast and relatively easy to measure and have been shown to provide reliable information on the chemical properties of biological materials. The KBr transmission technique is the most common tool for the quantitative estimation of lignin and suitable for routine work (Faix 1992). The diffuse reflectance infrared Fourier transform (DRIFT) method is suitable for a wood surface investiga-

tion and the lignin evaluation in wood, though its reproducibility is considered to be poor (Faix 1992). Absorption bands of spectrum represent vibration frequencies, which are characteristic of covalent bonds or functional groups and a whole molecule. The problem from the lignin modelling point of view is that many of the chemical components in wood contribute to the intensities at all or a large part of the wavenumbers, and that few wavenumber regions or bands, if any, thus reflect purely lignin (Ferraz et al. 2000, Costa e Silva et al. 1999); specifically, the aromatic signal intensities are low in comparison with the more polar polysaccharides. Consequently, it would seem prudent to base lignin content modelling on the intensity information on the whole spectrum. This is likely to result in a dimensionality problem, as a spectrum typically consists of intensities at some thousands of wavenumbers but not more than some dozens or hundreds wood samples can realistically be expected to be available for modelling.

In earlier studies, the relation between lignin content of a wood sample and a Fourier transform infrared (FTIR) spectra measured on it has been quite successfully modelled using linear regression in *Eucalyptus globulus* (Rodrigues et al. 1998), principal component regression (PCR) in *Picea sitchensis* and biodegraded *Pinus radiata* (Costa e Silva et al. 1999, Ferraz et al. 2000), partial least squares (PLS-1) in *Pinus radiata* (Meder et al. 1999), and projection to latent structures (PLS-2; lignin modelled simultaneously with glucan and polyoses) in biodegraded *Eucalyptus globulus* and *Pinus radiata* (Ferraz et al. 2000). The models have been built with a collection of individual wavenumbers or wavenumber regions or the whole spectrum as the input; the dimension reduction has then been tackled with a theoretically or empirically motivated selection of individual wavenumbers, or with principal component analysis (PCA), or, as in PLS-1 and PLS-2 methods, with a method resembling PCA where the components are formed by maximising not their variances but their covariances with the lignin content. The work by Gierlinger et al. (2002), although not dealing with lignin and FTIR but modelling heartwood extractives in *Larix* sp. with PLS models based on FTNIR spectra, sets a good example in coping with the various aspects

of sample preparation, spectrum preprocessing, wavenumber selection as well as model validation and evaluation that one is likely to encounter is this kind of modelling work. However, as far as we know, no such work has been done on Norway spruce. Also, although many articles have been written on the building of the statistical models, very few if any report the actual use of the obtained models for the purposes (e.g. screening) to which they were intended.

The purpose of our work was to develop a method based on FTIR spectra to determine the relative total lignin content of clonal Norway spruce samples and to use the method to study lignin variation in a large number of samples of similar kind. The aim was to replace the combination of three wet chemical methods (extraction, Klason lignin and acid soluble lignin determination) with one FTIR spectroscopic method, where the total lignin content is predicted in the small amount of powdered unextracted wood sample. This paper describes the building of an empirical lignin content model by using all-subset principal component regression; with the model, the percentage of dry mass of total lignin in a wood sample may be predicted by the FTIR spectrum measured on it. The key feature of the model building was that the selection of the final model was based equally on its fit in the estimation data and on its prediction performance in the test data. The paper also reports the application of the model to predict the lignin content variation in a large number of samples taken from the same three Norway spruce cutting clones planted on the same three sites of different fertility and climate from which the model was built.

2 Material and Methods

2.1 Material

Disks of wood were sawn at breast height (1.3 m) from the stems of 44 trees representing three different Norway spruce (*Picea abies* [L.] Karst.) cutting clones (A, B, C) growing at three different sites in Finland: Loppi (60°37'N 24°26'E), Imatra (61°08'N 28°48'E) and Kangasniemi (61°57'N 26°41'E). The trees were 26, 28 and 24 years old

at the time of felling. Samples (about 5 g) were taken from annual rings 3–6 in the heartwood and from three annual rings (rings between 13 and 22) in the middle of sapwood area. The distribution of the samples in different site-clone combinations is shown in Table 1. In addition, samples were taken from earlywood of 45 individual annual rings from 9 trees (5 samples per tree, 3 trees per each of the three clones) grown in one site (Loppi). Trees with the highest, average and slowest growth rate were chosen from each of the three clones. Annual rings with high and low peaks of weight density were further chosen in order to maximise density variation. The samples were taken avoiding knots and compression wood. The Norway spruce clones and their growth rate, weight density, mechanical strength properties and lignin modification experiments are described in Raiskila et al. 2006a, 2006b.

2.2 Klason Lignin and Acid Soluble Lignin Measurement

For model estimation and model testing, the relative total lignin (Klason lignin + acid soluble lignin) content was measured in duplicate for the sapwood and heartwood samples of 12 stems (24 samples) representing the three different clones growing in the three different sites (Table 1). Wood samples were ground frozen with a blade-mill (Polymix PX-A10). The dry solids content of the milled wood samples was determined at 103 °C. The samples of air-dried wood powders (3 g) were extracted with acetone, ethanol and water using a Soxhlet apparatus for 6 hours with each solvent separately (modified KCL 1982). After evaporation of the solvents the residues were dried at 103 °C, allowed to cool in a desiccator and then weighed. The amount of acid insoluble lignin was determined by the Klason method (KCL 1982, Dence 1992). The samples of the extracted wood powders (300 mg) were treated with 3 cm³ of 72% sulfuric acid in an ultrasonic bath for 1 hour. The mixtures were diluted with about 82 cm³ portions of water and autoclaved at 125 °C for 1 hour. The precipitates were collected with sintered glasses (4G) by suction filtration and washed with water. The sinters with the acid insoluble lignin (Klason lignin)

Table 1. Numbers of the samples taken from each site-clone combination and used for model estimation, model testing and lignin content prediction. The numbers of the stems are given in parentheses. In model estimation, model test and prediction data two samples (one from heartwood and one from sapwood) were taken in each stem. In tree ring prediction data five earlywood samples (each from a separate annual ring) were taken in each stem.

	Clone	Loppi	Imatra	Kangasniemi	Total
Model estimation data	A	2 (1)	2 (1)	2 (1)	6 (3)
	B	2 (1)	2 (1)	2 (1)	6 (3)
	C	2 (1)	2 (1)	2 (1)	6 (3)
	Total	6 (3)	6 (3)	6 (3)	18 (9)
Model test data	A		2 (1)		2 (1)
	B		2 (1)		2 (1)
	C		2 (1)		2 (1)
	Total		6 (3)		6 (3)
Prediction data	A	8 (4)	6 (3)	8 (4)	22 (11)
	B	8 (4)	6 (3)	8 (4)	22 (11)
	C	8 (4)	6 (3)	6 (3)	20 (11)
	Total	24 (12)	18 (9)	22 (11)	64 (32)
	Total	30 (15)	30 (15)	28 (24)	88 (44)
Tree ring prediction data	A	15 (3)			
	B	15 (3)			
	C	15 (3)			
	Total	45 (9)			

Table 2. Summary of the measured relative total lignin contents (Klason lignin + acid soluble lignin) in the model estimation data and model test data.

Data	Minimum	1st quartile	Median	Mean	3rd quartile	Maximum	Standard deviation
Estimation (n=18)	23.2	25.4	25.7	25.7	26.1	28.1	1.03
Test (n=6)	23.4	26.2	26.8	26.5	27.7	28.0	1.68

were dried at 103 °C, cooled in the desiccator and weighed. In order to determine the amount of acid soluble lignin the filtrates were diluted with water to 250 cm³. Absorption of the acid solutions with the dissolved lignin was measured at 203 nm using sulfuric acid of the same concentration as a blank (KCL 1982). The absorbance readings were obtained with a Shimadzu UV-2401 PC UV-VIS Recording spectrophotometer. The relative total lignin (Klason lignin + acid soluble lignin) content was calculated from the unextracted wood as follows: Klason lignin % = $p \times (100 - u) / m$, in which p = precipitate [g], u = extractives [%] and

m = calculated dry weight of extracted sample [g]. The acid soluble lignin content was calculated using a lignin absorptivity of 128 l g⁻¹ cm⁻¹ and corrected because of the absorption of carbohydrates according to a procedure of KCL (1982). The relative total lignin content of each sample was determined as the mean of the duplicate measurements; this is referred to as the *measured lignin content* of the sample. The variation of the measured lignin contents in the model estimation and model testing data sets is summarised in Table 2.

2.3 FTIR Analysis

For model estimation and model testing, FTIR analysis was performed in triplicate on the same 24 samples of 12 stems for which the relative total lignin (Klason lignin + acid soluble lignin) content was measured with the wet chemical methods. For lignin content prediction, single spectra were recorded on the heartwood and sapwood samples of the rest 32 stems (64 samples; Table 1) and in addition to this double spectra on the earlywood samples of annual rings (45 samples) from the 9 stems collected from one site (Loppi). Wood samples were ground frozen with a blade-mill (Polymix PX-A10), and half of the unextracted finely ground wood was passed through a sieve with hole size of 0.125 mm (see Faix and Böttcher 1992). Subsamples of 3.00–3.04 mg of sieved and unsieved wood powder were added to 300.0–301.0 mg of dry KBr in test tubes. The samples were dried at 60 °C for 2 hours, then cooled in a desiccator and mixed with a test tube mixer. The mixtures were pressed (112 bar/2 min) into discs with a diameter of 13 mm using a hydraulic press (Perkin Elmer, Hydraulische Presse) equipped with a vacuum pump. FTIR spectra were then recorded from the KBr tablets of the sieved and unsieved samples (3 tablets per sample for model estimation and testing, 1 tablet per sample or 2 tablets per earlywood samples for lignin content prediction) on a Perkin Elmer System 2000 FT-IR spectrometer (software version 4.0) equipped with a MIRTGS detector with a resolution of 4 cm⁻¹ using the transmission technique. Altogether 16 scans were accumulated from each sample. Data were acquired in the wavenumber range of 4000–500 cm⁻¹ (wavelength range of 2500–20 000 nm).

2.4 Principal Component Regression Modelling

Principal component regression (PCR), instead of the also commonly used partial least squares (PLS), was chosen as the modelling approach because it straightforwardly follows the standard statistical theory of linear models (as to estimation, testing and prediction; see e.g. Jolliffe 2002) and because it, unlike PLS, is easy to carry out

with any general statistical or matrix computation software. In general, PCR and PLS have been found in practice to give comparable results (Næs et al. 2002).

As already mentioned, the 24 samples from the 12 trees for building lignin content models were divided into two sets: model estimation data consisted of 18 samples from 9 trees, each tree representing one of the three clones growing in one of the three sites (Table 1), whereas model test data comprised 6 samples from 3 trees, each tree representing one of the three clones growing in one site (Imatra; Table 1).

Three different preprocessing methods (normalisations) were applied to the spectra recorded on the sieved and unsieved samples (Table 3). The normalisations were performed on each of the three repeated spectrum measurements of a sample, and the final normalised spectrum was then the pointwise average of these normalised replicates. Of the whole wavenumber range (4000–500 cm⁻¹), only the subrange of 1850–500 cm⁻¹ known to encompass lignin-related information (Hergert 1971) was eventually employed in the modelling. Alternatively, to diminish the effect of lignin-unrelated variation in intensity values, the modelling was performed on 13 subjectively selected wavenumber regions containing 299 wavenumbers (Table 3), the choice of which was based on chemical knowledge (Hergert 1971) and previous empirical work (e.g. Rodrigues et al. 1998, Costa e Silva et al. 1999). In Fig. 1, the wavenumber range of 1850–500 cm⁻¹ of the raw and LS-normalised spectra of the sieved samples in the model estimation data and model test data are shown.

PCR was carried out separately for each combination of the sieving, normalisation and wavenumber selection factor values (Table 3). There were altogether 16 factor value combinations resulting from two sieving methods, four normalisations (raw spectra included) and two wavenumber selection schemes. The first stage of PCR, the principal component analysis (PCA) for dimension reduction, was performed on the sample covariance matrix of the intensity variables (intensities at wavenumbers 1850–500 cm⁻¹ or at the 299 wavenumbers of the 13 wavenumber regions). The matrix was estimated from the samples in the estimation data set. The use of

Table 3. Factors affecting the quality of the spectral information that were tested in modelling. A set of 17 principal component regression models was built for each combination of the factor values resulting in 272 ($2 \times 4 \times 2 \times 17$) candidate models.

Stage	Factor	Value
Sample preparation	Sieving	Unsieved Sieved fraction of 125 μm
Spectrum measurement and preprocessing	Preprocessing ^{a)}	No normalisation, raw spectra (R) Line subtraction (LS) Standard normal variate normalisation (SNV) Standard normal variate normalisation in the range of 1800–500 cm^{-1} (SNV1350)
Modelling	Wavenumber selection ^{b)}	Wavenumber range of 1850–500 cm^{-1} 13 wavenumber regions

^{a)} LS: The baseline passing through the intensity values at wavenumbers 4000, 1929, 835 and 500 is subtracted from the spectrum, and the maximum intensity value is set to 1.5 SNV: The mean taken over all the intensity values is subtracted from the spectrum, and the mean-corrected spectrum is then divided by the standard deviation taken over all the intensity values

^{b)} 13 wavenumber regions: 1610–1590, 1520–1500, 1474–1444, 1434–1414, 1384–1364, 1341–1315, 1280–1260, 1233–1213, 1150–1124, 1043–1019, 870–850, 824–804, 628–608 cm^{-1} (299 wavenumbers in total)

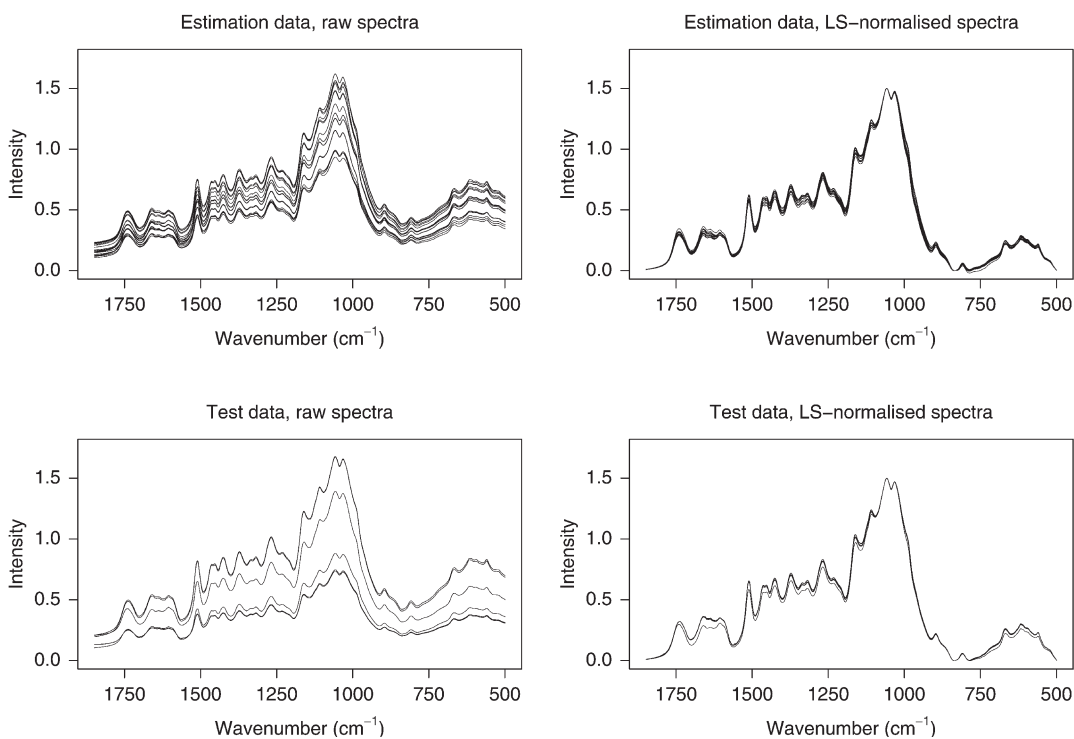


Fig. 1. Wavenumber range of 1850–500 cm^{-1} of the raw and LS-normalised (Table 3) spectra of the sieved samples in the model estimation data ($n=18$) and model test data ($n=6$). The final model was based on the LS-normalised spectra.

the covariance matrix was justified by the fact that the observed intensities in different samples were more or less at the same measurement scale. Thus, their variances were of similar magnitude, especially after the within-spectrum normalisation. Of the resulting principal components (PCs; uncorrelated linear combinations of the intensity variables), the 17 first ones accounting for a non-zero portion of the total variance of the intensity variables were retained in the analysis.

The retained PCs constituted the set of possible explanatory variables in an ordinary linear model with the measured relative total lignin content as the response variable and with the usual assumptions on the additive random error term, i.e. the error terms of different observations are independent and identically normally distributed with expectation 0 and constant variance. The parameters of the all-subset regressions were estimated with the ordinary least squares method (OLS) in the estimation data, i.e. all the possible combinations of the 17 retained PCs were tried as explanatory variables. In each model size class $p=1, 2, \dots, 17$, (i.e., among the models with p PCs as the explanatory variables), the model with the largest adjusted coefficient of determination (R^2_{adj}), or equivalently, with the smallest root mean square error (RMSE) was taken as a candidate model for the final model selection (for the definition of the concepts, see e.g. Weisberg 1985 and Table 4).

Note that in the parameter estimation the model assumption of mutually independent observations (samples) was clearly violated by the within-tree and within-plot dependencies of the estimation data. Such a flaw is not unusual in these kind of studies (see e.g. Meder et al. 1999), but it is usually passed without a mention. The violation causes the parameter estimate variances to become underestimated and, accordingly, the related significance tests to become too optimistic, whereas the parameter estimates themselves are still unbiased (Weisberg 1985). This can be regarded as acceptable for a prediction model.

As we were aspiring after a model for prediction, the selection among the $17 \times 16 = 272$ candidate models (17 models in each of the 16 factor value combinations) was based not only on the fit in the estimation data but also on the performance in the leave-one-out cross-validation in the

estimation data and, most importantly, on the prediction capability in the test data. Therefore, the root mean square error of lignin content in the estimation data (RMSE), the leave-one-out cross-validation estimate of root mean square prediction error in the estimation data (RMSPE_{CV}), and the root mean square prediction error in the test data (RMSPE) were computed for each candidate model and used as the model selection criteria (for the definition of the concepts, see e.g. Weisberg 1985 and Table 4). The criteria were plotted against the model size, and the most parsimonious models – to avoid over-fitting – with satisfying criteria values were chosen for further study. This involved 1) checking the model assumptions (normality of residuals with Q-Q plots, variance homoscedasticity of residuals with residual plots), 2) testing the significance of the parameter estimates (F-test for overall model significance, t-tests for individual parameters), 3) diagnosing the model fit (by means of plots of raw residuals, standardised residuals and studentised residuals) and 4) studying the influence of individual observations (leverages, and changes in regression coefficients, predicted values and RMSE that result from the deletion of each observation) in the customary manner (see e.g. Belsley et al. 1980, Weisberg 1985). The model with the best “overall performance” was chosen as the final model to be used for lignin content prediction in this study. Modelling computations were performed with S-Plus 3.4 and R 1.9.1 software (Venables and Ripley 1997, <http://www.r-project.org/>).

2.5 Lignin Content Prediction

With the final model, the relative total lignin content was predicted in 64 samples from 32 trees, 12 trees being taken from Loppi (4 trees per each of the three clones), 9 trees from Imatra (3 trees per each of the three clones), and 11 trees from Kangasniemi (4 trees per clones A and B, 3 trees per clone C; Table 1). To avoid extrapolation in the explanatory variables, the similarity of the normalised spectrum of each sample to the normalised spectra in the model estimation data was controlled with the Hotelling T^2 test based on the Mahalanobis distance between the sample and the estimation data centroid in the relevant PC-

space (Mardia et al. 1979). All the samples with the observed p -value larger than 0.05 were taken into the prediction (i.e., the samples accepting the null hypothesis that the sample point equals the estimation data centroid in the PC-space given the normality of distribution and the common covariance matrix estimated with the sample covariance matrix). In addition, the relative total lignin content was predicted in 45 earlywood samples (5 samples per tree) from 9 trees (3 trees per each of the three clones) from Loppi (Table 1).

A point prediction of the lignin content of a sample was obtained as the fixed part of the final model with the estimated parameter values. As usual, the PC scores used as the explanatory variable values were computed from the centered spectrum of the new sample; centering was done with the mean spectrum of the estimation data. The variance of the point prediction, the variance of the prediction error, and the 95% prediction interval based on the normality assumption were estimated in the usual manner (Weisberg 1985) using the RMSE and the inverse of the moment matrix of the model in the estimation data as well as the vector of the PC scores computed from the new sample spectrum as the input.

A Matlab macro was constructed to facilitate the use of the model for prediction. With a LS-normalised spectrum as the input, the macro outputs the point prediction, the estimated variance of the point prediction, the estimated variance of the prediction error, and the 95% prediction interval.

2.6 Statistical Analysis of Measured and Predicted Lignin Contents

The relative total lignin content measurements (24 samples from 12 trees) obtained with the wet chemical methods and predictions (64 samples from 32 trees) obtained with the final model were pooled into one data set. Differences in the amount of lignin in these data were analysed with the one-way analysis of variance (ANOVA) using a mixed model in the SPSS for Windows program version 12.0.1. The effect of clone (A, B, C) and growth site (Loppi, Imatra, Kangasniemi) on the heartwood and sapwood lignin content was tested pairwise at $p \leq 0.05$ level with a one-way

Tukey HSD^{a,b,c} test based on the normal distribution. The stem was used as a random factor. Differences in the predicted relative total lignin content of earlywood (45 samples from 9 trees) obtained with the final model were analysed with the one-way ANOVA and the effect of year on the earlywood lignin content was tested pairwise at $p \leq 0.05$ level with the Tukey HSD test. The results were considered at significance levels $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$.

3 Results

3.1 Principal Component Regression Modelling

In Fig. 2, the model selection criteria (RMSE, RMSPE_{CV}, RMSPE) are plotted as a function of model size (the number of PCs involved) for 213 of all the 272 candidate models considered; each point represents the model with the smallest RMSE in the particular size class and factor value combination, and models of larger size and with larger RMSE than the one with the minimum RMSE in the particular factor value combination are omitted. The figure very concretely exposes the trade-off between a good fit and a decent prediction capability: large models with many PCs tended to follow the estimation data too closely and thus predicted poorly in the slightly different test data. Especially the models based on unsieved samples seemed to be prone to this over-fitting. Irrespective of the preprocessing method applied, the models based on sieved samples and continuous wavenumber range 1850–500 cm⁻¹ showed the most balanced behaviour in terms of all the three model selection criteria, and therefore the attention was focused on this class of 54 models.

Due to the risk of over-fitting, models with more than 7–8 PCs in the chosen model class were considered unfeasible for prediction, even though they were performing quite well also in the test data (producing RMSPEs around 1.0%; Fig. 2). The set of possible candidates was hence reduced into 32 models with less than 9 PCs in them. The statistical quality of these models was examined, and many of the models proved to be

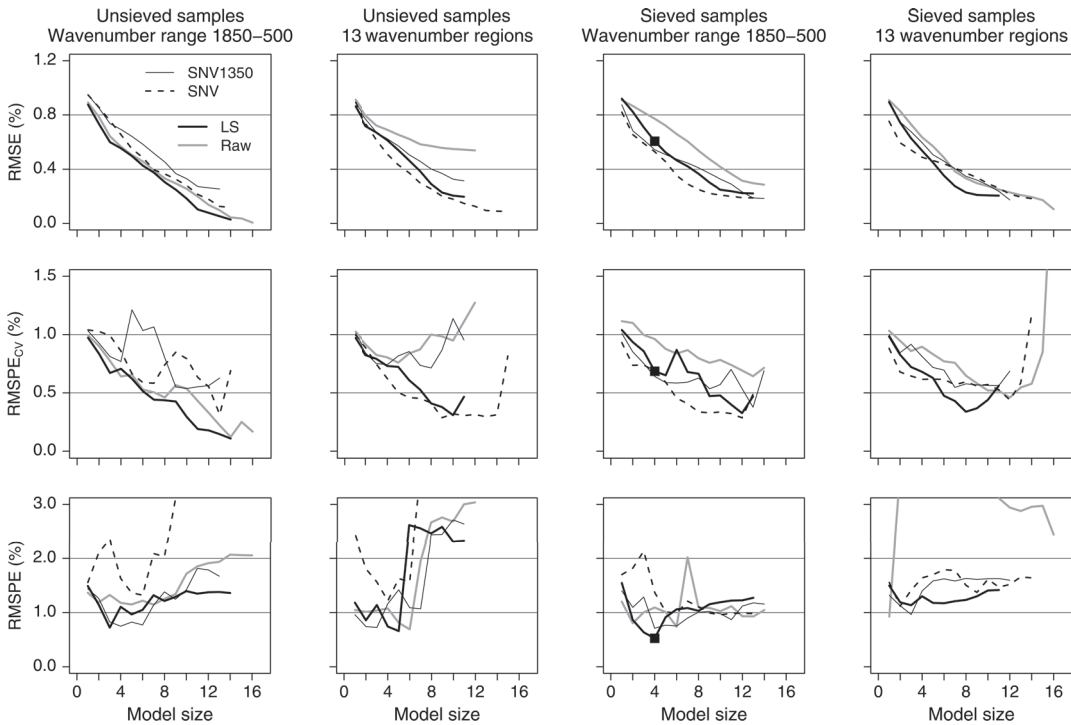


Fig. 2. Model selection criteria (RMSE, $RMSPE_{CV}$, $RMSPE$) with respect to model size (number of principal components included as the explanatory variables) in 213 of the 272 candidate models obtained with different wood powder sieving procedures, spectrum preprocessings and wavenumber selections (Table 3). RMSE is the root mean square error of lignin content in the estimation data ($n=18$), $RMSPE_{CV}$ is the leave-one-out cross-validation estimate of root mean square prediction error in the estimation data, and $RMSPE$ is the root mean square prediction error in the test data ($n=6$). Each point represents the model with the smallest RMSE in that size class. The model selected for use in prediction in this study (Table 4) is marked with a black square.

decent (with adequate normality and homoscedasticity of residuals, significant parameter estimates, “clean” residual plots etc.). The aim being a model for prediction, the role of the test data was emphasised in the final selection among the statistically adequate models: the model with the clearly smallest $RMSPE$ in the test data (Fig. 2) was chosen as the final model. This decision meant dismissing several adequate models fitting better to the estimation data but predicting worse in the test data. The Hotelling T^2 test showed ($p=0.0747$) that the test data, although collected from only one site (Imatra), does not significantly (at 0.05 risk level) deviate from the estimation data in the 4-dimensional PC-space associated to the model (given the normality assumption and

the common estimated covariance matrix); thus the prediction error in the test data is a reasonable selection criterion when the model is intended to be applied to similar kind of data.

The final model is summarised in Table 4. Note that the first PC accounting for 86% of the total variance of the intensity variables in the estimation data was not included in the model, which indicates that most of the spectral variation between the samples was due to chemical properties unrelated with the lignin content. The coefficients of the intensity variables in the 4 PCs included in the model are presented in Fig. 3. The model fit in the estimation data and the prediction performance in the test data are illustrated in Fig. 4; following the common statistical terminol-

Table 4. Summary of the principal component regression model chosen for lignin content prediction in this study. The model was estimated from the wavenumber range of 1850–500 cm⁻¹ of the LS-normalised (Table 3) spectra of the sieved samples and involves the principal components (PC) that accounted for the 2nd (8.9%), 3rd (2.0%), 4th (1.3%) and 6th (0.3%) largest portions of the total variance of the intensity variables (intensities at wavenumbers 1850–500 cm⁻¹) in the estimation data.

$$\text{Model: lignin content}^{\text{a)}} = \beta_0 + \beta_1\text{PC2} + \beta_2\text{PC3} + \beta_3\text{PC4} + \beta_4\text{PC6} + \text{random error}^{\text{b)}})$$

Parameter	Estimate	Standard error	p-value of t-statistic	
β_0	25.681	0.143	0.0000	
β_1	2.046	0.575	0.0036	
β_2	-3.049	1.231	0.0277	
β_3	-4.367	1.496	0.0120	
β_4	9.802	3.240	0.0098	
F-statistic (df 4 and 13)	9.100	p-value of F-statistic	0.000986	
RMSE (estimation data)	0.606%	R ² (estimation data)	0.737	
RMSPE _{CV} (estimation data)	0.686%	R ² _{adj} (estimation data)	0.656	
RMSPE (test data)	0.525%	R ² _{CV} (estimation data)	0.702	
		R ² _P (test data)	0.903	

RMSE = $[\text{SSE}/(n-p)]^{1/2}$, where SSE = sum of squared residuals, $n = 18$, $p = 5$

RMSPE_{CV} = $[\text{SSE}_{\text{CV}}/n]^{1/2}$, where SSE_{CV} = sum of squared prediction errors in leave-one-out cross-validation, $n = 18$

RMSPE = $[\text{SSE}_P/m]^{1/2}$, where SSE_P = sum of squared prediction errors, $m = 6$

R² = $1 - \text{SSE}/\text{SST}$, where SST = sum of squared differences between lignin content in each sample and mean lignin content over the samples

R²_{adj} = $1 - [\text{SSE}/(n-p)]/[\text{SST}/(n-1)] = 1 - \text{RMSE}^2/\text{Var}(\text{lignin content})$

R²_{CV} = $1 - \text{SSE}_{\text{CV}}/\text{SST}$

R²_P = $1 - \text{SSE}_P/\text{SST}$

^{a)} Relative total lignin content: proportion of total lignin of dry mass, expressed in percentage

^{b)} Assumptions on the random error needed in parameter estimation and statistical testing: normally distributed with expectation 0 and variance σ^2 , errors of different observations mutually independent. RMSE² in the estimation data is an unbiased estimate of σ^2 .

ogy, the lignin content obtained with the model is termed *estimated lignin content* for the samples in the model estimation data and *predicted lignin content* for the samples in the independent model test data.

The residual plots of the model (Fig. 5) reveal one deviating sample in the estimation data with a negative residual larger than 1% in absolute value and with standardised and studentised residuals below -2. However, this heartwood sample from the clone A growing in Loppi could not be categorised as an outlier: its spectrum did not significantly differ from the centroid of the other model estimation data in the 4-dimensional PC-space, nor was its measured lignin content (25.07%) extreme. Fortunately, the leverage of the observation was low (0.146), and its removal

did not markedly influence the model parameter estimates, RMSE or the predicted lignin value.

3.2 Lignin Content Prediction

All the 64 samples from the 32 trees in the prediction data were accepted for the prediction, as their Mahalanobis distances from the model estimation data centroid were small enough not to result in similarity hypothesis rejection in Hotelling T² test at 0.05 risk level. The different sites did not considerably differ from each other in terms of the distribution of the Mahalanobis distances.

As is evident from the formulae of the variance of the point prediction (which incorporates the effect of the error in the parameter estimates of

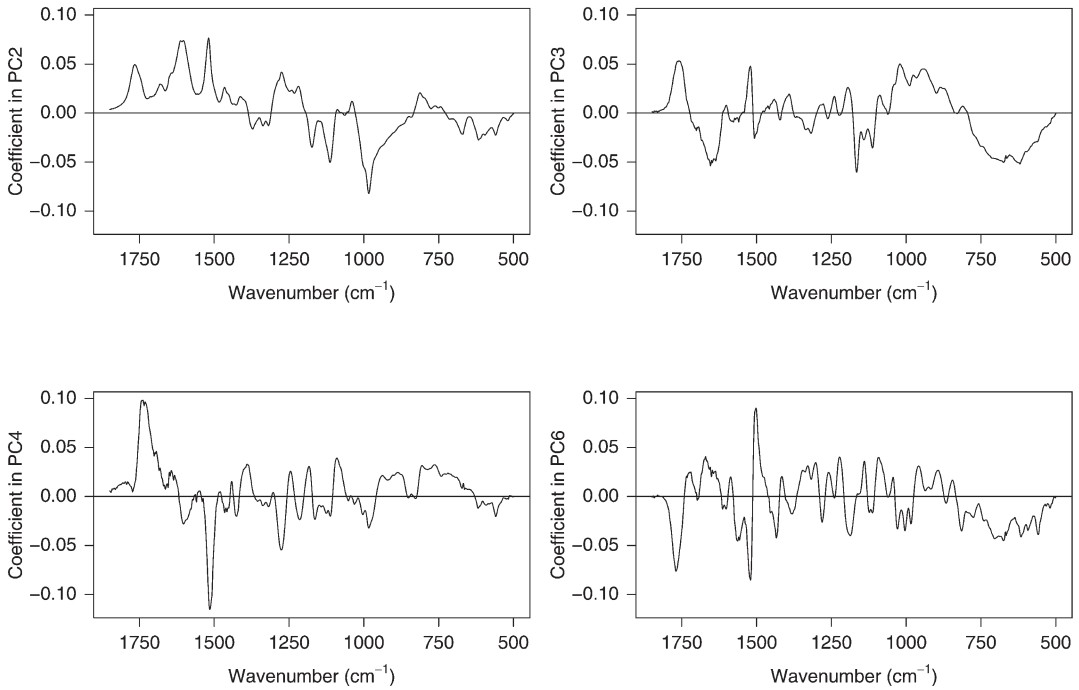


Fig. 3. Coefficients of the intensity variables (intensities at wavenumbers 1850–500 cm⁻¹) in the four principal components (PC) included in the final model (Table 4); the PCs were estimated from the wavenumber range of 1850–500 cm⁻¹ of the LS-normalised spectra of the sieved samples in the model estimation data (n=18) (Fig. 1, Table 3).

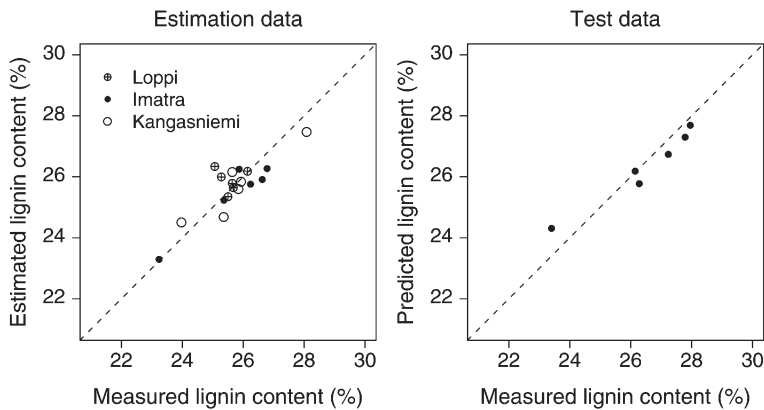


Fig. 4. Relative total lignin contents estimated/predicted with the final model (Table 4) versus the measured (Klason lignin + acid soluble lignin measurement) values in the model estimation data (n=18) and model test data (n=6).

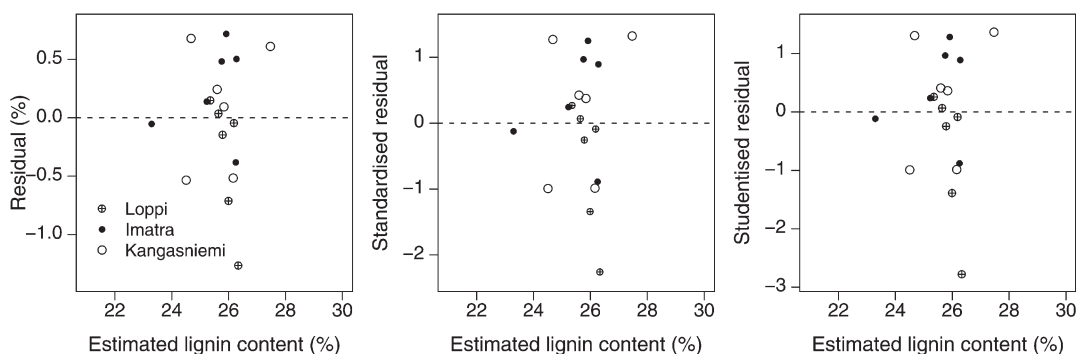


Fig. 5. Residual plots of the final model (Table 4) in the model estimation data (n=18).

Table 5. Summary of the prediction results in the 64 samples of the prediction data.

	Minimum	1st quartile	Median	Mean	3rd quartile	Maximum	Standard deviation
Predicted relative total lignin content (%)	23.2	25.3	25.8	25.8	26.6	28.5	0.97
Standard error of prediction (%)	0.19	0.65	0.87	0.88	1.12	1.45	0.31
Standard error of prediction error (%)	0.64	0.89	1.06	1.08	1.28	1.57	0.24

the linear model) and the variance of the prediction error (which incorporates also the effect of the random error of the model and is employed in prediction interval construction; Weisberg 1985), the spectral dissimilarity of a sample to the model estimation data increased the estimated uncertainty of the lignin content prediction (Fig. 6b). It did not, however, systematically influence the level of the prediction (Fig. 6a). Consequently, the uncertainty related to the lignin content prediction was independent of the level of the prediction, that is, large lignin contents were not predicted less precisely than small ones or vice versa (Fig. 6c).

The predicted lignin contents were realistic with no highly deviating values and with the range and the variation of the same magnitude as in the model estimation data (Table 5; cf. Table 2). Also the estimated precision of the prediction can be regarded as satisfactory with 40/64 samples having the prediction standard error less than 1% and 45/64 samples having the standard error of the prediction error smaller than 1.25% (corresponding to 2.5% approximate prediction interval).

3.3 Variation in Measured and Predicted Lignin Content

In Fig. 7a, the variation in the relative total lignin content in the combined data set consisting of measurements (24 samples from 12 trees) and predictions (64 samples from 32 trees) is presented with respect to site, clone and sample location (heartwood, sapwood). The heartwood lignin content varied from 24.80% to 28.48% and sapwood lignin content from 23.17% to 27.79% between the individual stems in this material. According to the ANOVA, the effects of site, clone and site-clone interaction on the amount of sapwood lignin were statistically significant at risk levels of 0.001, 0.01 and 0.01, respectively (Table 6a). The differences in the sapwood lignin contents between the sites and between the clones were tested pairwise at $p \leq 0.05$ level (Table 6b). The fertile site in Loppi produced rapidly-grown wood in which the mean sapwood lignin content ($26.31 \pm 0.62\%$, \pm SD between stems) was significantly higher than that of wood grown in Imatra ($24.91 \pm 1.31\%$) and in Kangasniemi ($25.01 \pm 0.60\%$). The lowest sap-

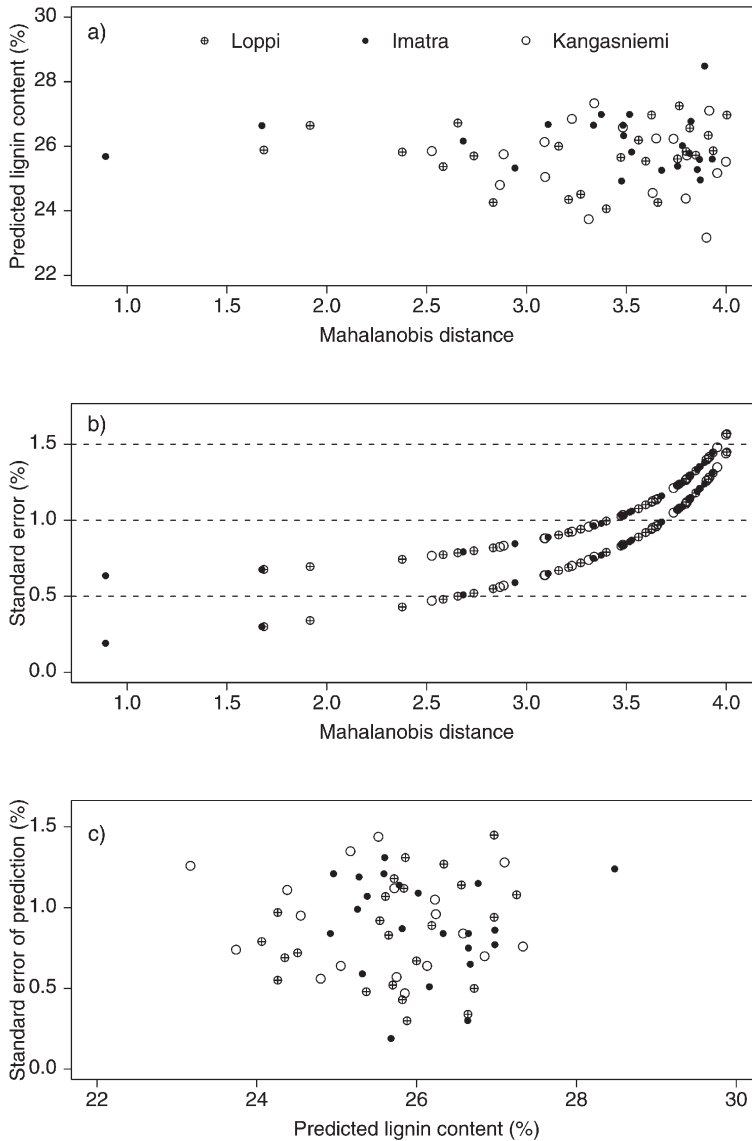


Fig. 6. Prediction performance of the final model in the prediction data (n=64): a) the predicted relative total lignin content versus the Mahalanobis distance of the sample from the model estimation data centroid in the 4-dimensional PC-space, b) the estimated uncertainty of the prediction expressed with the standard error of the prediction (lower set of points) and with the standard error of the prediction error (upper set of points) versus the Mahalanobis distance, and c) the standard error of the prediction versus the predicted relative total lignin content for the 64 samples in the prediction data.

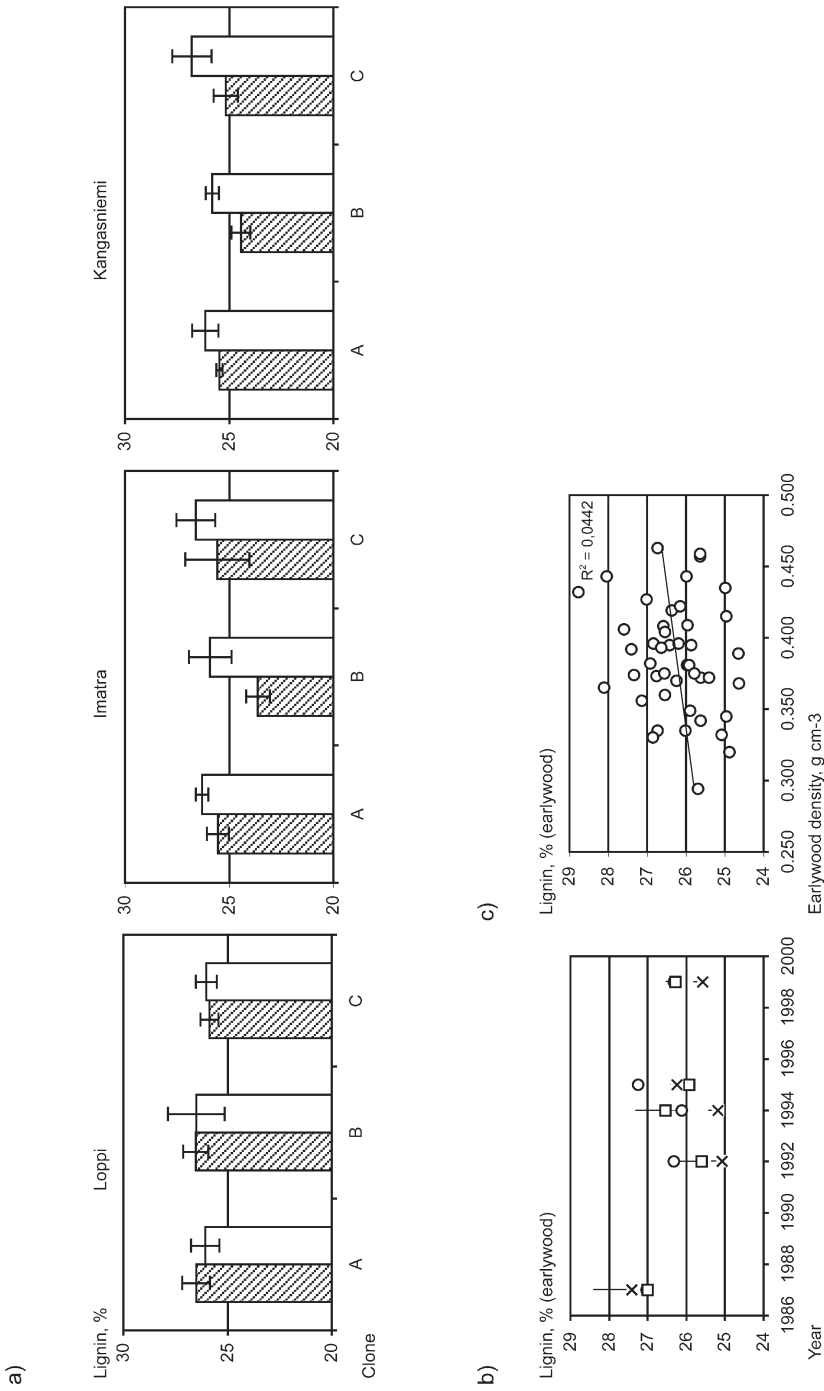


Fig. 7. a) Combined measured and predicted relative total lignin contents (\pm SD) in the sapwood (grey column) and heartwood (white column) of clones A, B and C from Loppi, Imatra and Kangasniemi. b) Predicted relative total lignin content (\pm SE) in the earlywood of clones A (O), B (□) and C (X) versus the year in Loppi. c) Predicted relative total lignin content of earlywood versus the weight density in Loppi.

Table 6. Statistical analysis of the combined data of measured and predicted relative total lignin contents in the clones (A, B, C) growing in Loppi, Imatra and Kangasniemi and the predicted relative total lignin contents of earlywood in trees grown in Loppi. a) Analysis of variance and b) pairwise comparison. The statistically significant differences ($p \leq 0.05$) between the clones, between the sites and between the annual rings have been marked with a and b. Ring 1 = year 1999, 2 = 1995, 3 = 1994, 4 = 1992 and 5 = 1987.

6a)				
	F values			
	Heartwood lignin	Sapwood lignin	Earlywood lignin	
Intercept	45680.722 ***	56311.189 ***		
Site	0.031	17.845 ***		
Clone	0.928	7.517 **		
Site-clone	1.115	4.578 **		
Year			4.536 **	

$p \leq 0.05$ *, $p \leq 0.01$ ** and $p \leq 0.001$ ***

6b)				
	Lignin % (SD)			
	Heartwood	Sapwood	Earlywood	
Clone A	26.17 (0.53) a	25.84 (0.68) b	Ring 1	26.06 (0.49) b
Clone B	26.08 (0.98) a	24.86 (1.37) a	Ring 2	26.47 (0.76) ab
Clone C	26.45 (0.82) a	25.56 (0.97) b	Ring 3	25.95 (0.98) b
Loppi	26.19 (0.89) a	26.31 (0.62) a	Ring 4	25.67 (0.78) b
Imatra	26.27 (0.82) a	24.91 (1.31) b	Ring 5	27.14 (0.93) a
Kangasniemi	26.21 (0.73) a	25.01 (0.60) b		
Mean	26.23 (0.80)	25.42 (1.10)	Mean	26.26 (0.93)
Min.	24.80	23.17	Min.	24.64
Max.	28.48	27.79	Max.	28.77
Median	26.01	25.53	Median	26.20

wood lignin content ($24.86 \pm 1.37\%$) was found in the clone B but depending on the site the clone B showed more variation than the clones A and C the sapwood lignin contents of which were $25.84 \pm 0.68\%$ and $25.56 \pm 0.97\%$, respectively. The mean lignin content was slightly higher in the heartwood ($26.23 \pm 0.80\%$) than in the sapwood ($25.42 \pm 1.10\%$) but there were not statistically significant differences in the heartwood lignin content between the sites and between the clones. The random factor had significant ($p \leq 0.001$) influence on both the heartwood and sapwood lignin because of the large variation between the individual stems.

Variation in the predicted relative total lignin content of earlywood (45 samples from 9 trees) in one site (Loppi) is presented with respect to clone and year in Fig. 7b. The earlywood lignin

content varied from 24.64% to 28.77% between the selected annual rings. The average earlywood lignin content was $26.26 \pm 0.93\%$ (\pm SD between rings). The annual variation in the amount of earlywood lignin was significant at the risk level of 0.01 (Table 6a). The differences in the earlywood lignin contents between the annual rings were tested pairwise at $p \leq 0.05$ level (Table 6b). The mean earlywood lignin content of annual ring 5 ($27.14 \pm 0.93\%$) in the heartwood was significantly higher than that of the rings 1, 3 and 4 ($26.06 \pm 0.49\%$, $25.95 \pm 0.98\%$, $25.67 \pm 0.78\%$) in the inner and outer sapwood. The earlywood density of annual rings studied varied between 0.294–0.463 g cm⁻³ and the annual ring width between 1.3–5.4 mm (Raaskila et al. 2006b). No correlation was found between the lignin content and density of earlywood. (Fig. 7c).

4 Discussion

4.1 Modelling

Type of spectrum (transmission vs. diffuse reflectance) together with sample preparation is known to influence the reproducibility of the spectral measurements and the discernibility of the lignin-related variation in the spectra (see e.g. Faix and Böttcher 1992, Martens and Næs 1989). In this study, transmission spectra were used because of their better quality in this setting; also diffuse reflectance spectra were tried on both solid and KBr-mixed milled samples, but their quality was found too variable. In order to avoid extraction, which is the most laborious part of the wet chemical methods, spectra were recorded on unextracted samples; on the other hand, the amount of extractives in spruce wood is known to be fairly low (Saranpää 2002). Normalisation of spectra is in spectroscopy considered necessary for quantitative analysis and may markedly affect the results of the analysis (see e.g. Gierlinger et al. 2002). The methods may be divided into within-spectrum normalisations that utilise only the information in the spectrum itself and between-spectra normalisation that endeavour to harmonise a set of spectra from different samples; in both categories, the normalisations may be based on some reference bands or on the whole spectrum. Of the many of methods available, two such simple within-spectrum normalisations were chosen that can be applied entirely automatically (they do not, for example, require a possibly complicated and therefore often manually performed recognition of intensity differences between local minima and maxima (“peak heights”) as the normalisation used by Rodrigues et al. 1998).

Although using combinations of intensity values at only a few individual wavenumbers (bands) has sometimes been found to produce well-fitting lignin content models (Costa e Silva et al. 1999, Rodrigues et al. 1998), we considered it safer to employ large, often connected, parts of spectra (following e.g. Ferraz et al. 2000 and Meder et al. 1999) as they contain not only “pure” lignin-related information but also that masked by other major wood constituents. In our study, restricting the range of 1350 wavenumbers ($1850\text{--}500\text{ cm}^{-1}$) to the supposedly more lignin-related 13 regions

containing 299 wavenumbers (Table 3) did not improve the fit but made the prediction performance with respect to model size more variable and more dependent of the preprocessing method (Fig. 2). Automated methods for wavenumber range selection have been proposed (Westad and Martens 2000), but they are somewhat heuristic and apparently still need to be complemented with some manual selection (e.g. Gierlinger et al. 2002) and were not therefore considered in this study.

In PLS, where the uncorrelated principal components are formed by maximising the covariance between the lignin content and the linear combinations of the intensity variables, model selection means just deciding the number of components to be included in the model. In PCR, model selection is more complicated: if the components are straightforwardly taken in the order of their accounted variance of the intensity variables, then also components with little explanatory power on the lignin content risk being included (this was probably the case in the PCR models of Ferraz et al. 2000). Therefore all-subset regression was carried out in this study and only models with all the parameters deviating statistically significantly from zero were taken into consideration. Characteristically, the first PC accounting for most variance was not included in the final model. Model selection for prediction is a compromise between fit in estimation data and prediction capability in (independent) test data, which unknown future data are assumed to closely resemble. We chose to emphasise the role of the test data in the model selection, and as a result several candidate models fitted far better to the estimation data but none predicted in the test data as well as the one that was finally selected (Fig. 2).

Comparison of the results to those of some previous studies (Costa e Silva et al. 1999, Ferraz et al. 2000, Meder et al. 1999 and Rodrigues et al. 1998) was somewhat complicated by methodological problems: Model structure (number of components) in PLS models was sometimes allowed to change in cross-validation (Ferraz et al. 2000) or in test set with measured lignin content values (Gierlinger et al. 2002). It is evident that such exercises provide hardly any information on the validity of the original models; it is also unclear what structure would then be used in prediction

when no measured lignin contents are available. For no obvious reason, PCR models were also sometimes estimated without the intercept term, which resulted in non-zero means of residuals referred to as “average prediction error” (in Costa e Silva et al. 1999 this was 0.89% and in Ferraz et al. 2000 0.3%). If the intercept is included, as in the models of this study, the residuals always sum to zero. Treating replicate spectral measurements as independent observations (Ferraz et al. 2000), and using the magnitude of F-test statistic (or the corresponding p-value) as support to the acceptance of a H_0 hypothesis (Ferraz et al. 2000), although the distribution of the statistic is defined on the condition that H_0 is true, are some further examples of methodological problems.

Comparable results from the models of the previous studies mentioned above are collated in Table 7. Differences in material naturally set limit to comparisons: none of the studies dealt with Norway spruce, and one of them was based on biodegraded material. In terms of RMSE, the fit of our model in the estimation data appeared fairly similar to those in the other studies; proportioned to the lignin content variation in the estimation data, however, the random error variation was seen to be of a larger magnitude in our model than in the other studies, which was reflected in the lower R^2 value. (Note that 60 of our 272 candidate models had R^2 larger than 0.95 in the estimation data, but their prediction performance in terms of especially RMSPE was judged far poorer than that of the final model). Only Meder et al. (1999) performed leave-one-out cross-validation; they reported results rather similar to ours, although their models fitted slightly better to their estimation data. Rodrigues et al. (1998) were the only ones to use independent test data: although superior in the fit, their model appeared to equal our model in the prediction performance. Only Costa e Silva et al. (1999) applied their model to the independent prediction data of 83 samples with no lignin content measurements; they reported remarkably uniform standard errors of prediction between 0.86–0.97% (mean 0.89%, standard deviation 0.030 %), the uniformity probably stemming partly from the replicate nature of the data and partly from the small number of intensity variables (6) involved in the model; on average, our model predicted with similar mag-

nitude of estimated uncertainty (Table 4), but the variation on standard errors of prediction was far larger, apparently due to the far larger amount of spectral information incorporated in the model.

The model estimation and test data sets of this study were rather small, although not out of line with most of the other similar studies (Table 7). This naturally limits the range of usage of this kind of empirical model, which is not, however, a serious defect from our point of view: we did not pursue large variation in lignin content or in other chemical properties of the samples, because we only wanted to build a model for prediction in a limited kind of clonal data, that is, the model was intended to be applied only to samples that can be regarded very similar to those in the estimation data. The empirical modelling procedure presented here is, however, applicable to all kind of wood material, and a similar model could easily be built for e.g. natural or biodegraded samples.

4.2 Variation in Lignin Content

In this study Norway spruce trees from Loppi showed a higher sapwood lignin content than trees from Imatra or Kangasniemi. This may be due to the higher growth rate in Loppi (Raikila et al. 2006b). The clone B the sapwood lignin content of which was the lowest had the slowest growth rate. The lignin content (23.17–27.79%) is slightly less than reported values for Norway spruce (27.5–28.9%) (Brolin et al. 1995, Anttonen et al. 2002). The lignin content has been found to be affected by the growth rate of trees e.g. in a fertilisation test (Anttonen et al. 2002). The lignin content is influenced by the growth rate possibly because of the changes in the relative amounts of cellulose rich secondary layers of cell wall and highly lignified middle lamella and by the relative amounts of earlywood and cellulose rich latewood (Anttonen et al. 2002). The purpose of cloning in the 1970's was to increase the growth rate of trees. The three cutting clones (A, B, C) chosen for study were genetically uniform material and have grown in the different environments. The growth sites Loppi and Imatra were nutrient rich old agricultural lands and Kangasniemi was a medium fertile *Myrtillus*-type forest (Cajander 1926). The growth rate and wood properties of the

Table 7. Comparison of the final model to the models built in some other studies.

Study	Species	Spec- trum type ¹⁾	Model type	Spectral range (cm ⁻¹)	Estimation (calibration) data				Test (validation) data					
					n	Lignin range, st.dev. (%)	RMSE (%)	R ²	RMSPE _{cv} (%)	R ² _{cv}	m	Lignin range, st.dev. (%)	RMSPE (%)	R ² _p
This study	<i>Picea abies</i> (clonal)	TR	PCR	1850–500	18	[23.2, 28.1], 1.0	0.61	0.74	0.69	0.70	6	[23.4, 28.0], 1.7	0.53	0.90
Costa e Silva et al. (1999)	<i>Picea sitchensis</i> (clonal)	TR	PCR ^{a)}	1600–400	15	[24, 34], 2.9	0.89 ^{b)}	0.93						
Ferraz et al. (2000)	<i>Pinus radiata</i> (decayed)	DR	PCR	4000–824	42 ^{c)}	[23.3, 30.9], 2.0	0.63 ^{d)}	0.91	(1.06) ^{e)}	(0.39) ^{e)}				
	<i>Eucalyptus globulus</i> (decayed)	DR	PLS-2	4000–824	26 ^{c)}	[20.6, 27.8], 2.1	0.63 ^{d)}	0.93						
Meder et al. (1999)	<i>Pinus radiata</i> (clonal)	DR	PLS-1	2000–550	76	[24.4, 32.4] ^{f)} , 1.6	0.58	0.87	0.81	0.74 ^{g)}				
Rodrigues et al. (1998)	<i>Eucalyptus globulus</i> (provenance trial)	TR	Linear regression ^{b)}	1800–800	20	[23.3, 34.5], 2.7	0.44	0.98	0.91	0.67 ^{g)}	20	[23.7, 31.8], 2.2	(0.37) ^{h)}	(0.97) ⁱ⁾

a) Principal components formed from six ratios of raw intensities I(i)/I(1374 cm⁻¹), i = 1511, 1423, 1268, 1158, 1059, 1031 cm⁻¹.

b) Not reported in the study; computed from the reported residual standard deviation by the authors of this study.

c) Duplicate spectrum measurements from 21 or 13 actual samples considered as separate observations.

d) Not reported in the study; computed from the reported R² and variance of measured lignin by the authors of this study.

e) Not reported in the study; computed from the leave-three-out cross-validation performed in the study, therefore not directly comparable to RMSPE_{cv} and R²_{cv}.

f) Estimated from a figure.

g) Not reported in the study; computed from the reported RMSPE_{cv} and variance of measured lignin by the authors of this study.

h) Y = 10.7 + 76.3 [I(1505 cm⁻¹)/I(1157 cm⁻¹)], where Y is lignin content and I(1505 cm⁻¹) and I(1157 cm⁻¹) are scaled intensities.

i) Computed from the model $Y_{meas} = \alpha_0 + \alpha_1 Y_{pred}$, where Y_{meas} is the lignin content measured with acetyl bromide method and Y_{pred} is the lignin content predicted with the original linear regression model (see b)). In the model estimation data, the residuals $Y_{meas} - Y_{pred}$ coincide with the residuals $Y_{meas} - Y_{pred}$ of the original model and thus give the same RMSE (because the number of the parameters happens to be the same in both the models) and R² as the residuals of the original model; in the test data, however, the residuals $Y_{meas} - Y_{pred}$ do not coincide with the prediction errors $Y_{meas} - Y_{pred}$ of the original model, and therefore these characteristics do not correspond to RMSPE and R²_p. They should only be compared to the similarly obtained quantities in this study, namely to 0.39% ("RMSPE") and 0.96 ("R²_p").

j) TR = transmission, DR = diffuse reflectance.

clones are described in Raiskila et al. 2006b.

In this study the effects of site, clone and site-clone interaction on the amount of sapwood lignin were significant but not on the amount of heartwood lignin. The variation in the heartwood and sapwood lignin between individual stems was high. Several biotic and abiotic factors affect the growth of trees and thus, even the trees belonging to the same clone showed a large variation in lignin content. The mean lignin content of heartwood (26.23%) was slightly higher than that of sapwood (25.42%) and our results are in accordance with earlier results. The lignin content has been reported to decrease significantly in the radial direction from heartwood (28.3%) to sapwood (27.7%) and to be the lowest in the transition zone (27.3%) (Bertaud and Holmbom 2004). In earlier studies with 1-year-old plants and 9-year-old trees the lignin content did not vary significantly within and between full-sib families but was higher in trees than in plants and a standard error for the trees was lower than for the plants (Wadenbäck et al. 2004). The amount of lignin is also affected by the reaction wood formation (Barnett and Jeronimidis 2003) and the 'pseudo lignification' during heartwood formation (Magel 2000).

The annual variation in the amount of earlywood lignin was significant. The lignin content (24.64–28.77%) is slightly higher than reported values for earlywood (23–24%) (Gindl and Grabner 2000). In the earlier studies with Norway spruce the earlywood lignin content (32.2%) has been found to be significantly higher than the latewood lignin content (29.8%) but no clear differences between the annual rings were observed, however they studied only one stem (Bertaud and Holmbom 2004). One reason for the high variation between rings could be the selection of annual rings with very high and low weight density and variable ring width. However the earlywood lignin content did not correlate with the earlywood density. Also the annual variation in the growth and the weight density has been found to be significant and the growth increments did not correlate linearly with the weight density in this rapidly-growing clone material (Raiskila et al. 2006b).

5 Conclusions

A PCR-based method for predicting the relative amount of total lignin in clonal Norway spruce wood from FTIR transmission spectra was developed. Using some modelling practices (all-subset regression; model selection based on combination of RMSE and RMSPE_{CV} in the estimation data and RMSPE in the test data) that, despite being standard in statistics, have not been frequently applied in FTIR or NIR modelling studies, a model with no over-fitting in the estimation data and good prediction performance in the test data was obtained. For a set of samples representing the same three clones growing in the same three sites as the samples in the modelling data, the model was seen to produce realistic lignin content predictions with satisfactory estimated precision.

By the analysis of the model estimation and test data pooled with the prediction data, site, clone and site-clone interaction were found to have a significant effect on sapwood lignin content. The model was also used to predict the lignin content in the earlywood of 45 individual annual rings; by the analysis of these predictions, the annual variation in the amount of earlywood lignin was significant and the variation between individual stems was large.

The method requires only a simple sample preparation, and once the spectra have been recorded, it is fast and simple to use. A similar model, easily built by following the presented modelling procedure, could prove very advantageous when the natural genetic variation in lignin contents or variation caused by growth rate is determined e.g. in the extensive native stands of Norway spruce.

Acknowledgements

The Academy of Finland is gratefully acknowledged for the financial support of a programme on Sustainable Use of Forest Resources (Grant no: 52773). The authors thank Ph.D. Matti Sarén for his help with Matlab macro construction and Ph.D. Riikka Piispanen for her help with the statistical analysis. We thank Ms. Irmeli Luovula, Mr. Tapio Nevalainen and Mr. Tapio Järvinen for skilful technical help.

References

- Anttonen, S., Manninen, A.-M., Saranpää, P., Kainulainen, P., Linder, S. & Vapaavuori, E. 2002. Effects of long-term nutrient optimisation on stem wood chemistry in *Picea abies*. *Trees* 16: 386–394.
- Barnett, J.R. & Jeronimidis, G. 2003. Reaction wood. In: Barnett, J.R. & Jeronimidis, G. (eds.). *Wood quality and its biological basis*. Blackwell Publishing Ltd., Oxford. p. 118–136.
- Belsley, D.A., Kuh, E. & Welsch, R.E. 1980. *Regression diagnostics: identifying influential data and sources of collinearity*, John Wiley & Sons, New York. 292 p.
- Bertaud, F. & Holmbom, B. 2004. Chemical composition of earlywood and latewood in Norway spruce heartwood, sapwood and transition zone wood. *Wood Science and Technology* 38: 245–256.
- Brolin, A., Norén, A. & Ståhl, E.G. 1995. Wood and pulp characteristics of juvenile Norway spruce: a comparison between a forest and an agricultural stand. *Tappi J* 78(2): 203–214.
- Cajander, A.K. 1926. The theory of forest types. *Acta Forestalia Fennica* 29(3). 108 p.
- Costa e Silva, J., Nielsen, B.H., Rodrigues, J., Pereira, H. & Wellendorf, H. 1999. Rapid determination of the lignin content in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) wood by Fourier transform infrared spectrometry. *Holzforschung* 53: 597–602.
- Dence, C.W. 1992. The determination of lignin. In: Lin, S.Y. & Dence, C.W. (eds.). *Methods in lignin chemistry*. Springer-Verlag, Heidelberg. p. 33–61.
- Faix, O. 1992. Fourier transform infrared spectroscopy. In: Lin, S.Y. & Dence, C.W. (eds.). *Methods in lignin chemistry*. Springer-Verlag, Heidelberg. p. 83–109.
- & Böttcher, J.H. 1992. The influence of particle size and concentration in transmission and diffuse reflectance spectroscopy of wood. *Holz als Roh- und Werkstoff* 50: 221–226.
- Ferraz, A., Baeza, J., Rodriguez, J. & Freer, J. 2000. Estimating the chemical composition of biodegraded pine and eucalyptus wood by DRIFT spectroscopy and multivariate analysis. *Bioresource Technology* 74: 201–212.
- Fukushima, R.S. & Hatfield, R.D. 2001. Extraction and isolation of lignin for utilization as a standard to determine lignin concentration using the acetyl bromide spectrophotometric method. *Journal of Agricultural and Food Chemistry* 49: 3133–3139.
- Gierlinger, N., Schwanninger, M., Hinterstoisser, B. & Wimmer, R. 2002. Rapid determination of heartwood extractives in *Larix* sp. by means of Fourier transform near infrared spectroscopy. *Journal of Near Infrared Spectroscopy* 10: 203–214.
- Gindl, W. & Grabner, M. 2000. Characteristics of spruce [*Picea abies* (L.) Karst.] latewood formed under abnormally low temperatures. *Holzforschung* 54: 9–11.
- Hatfield, R. & Fukushima, R.S. 2005. Can lignin be accurately measured? *Crop Science* 45: 832–839.
- , Grabber, J., Ralph, J. & Brei, K. 1999. Using the acetyl bromide assay to determine lignin concentrations in herbaceous plants: some cautionary notes. *Journal of Agricultural and Food Chemistry* 47: 628–632.
- Hergert, H.L. 1971. Infrared spectra. In: Sarkanen, K.V. & Ludwig, C.H. (eds.). *Lignins; occurrence, formation, structure and reactions*. Wiley-Interscience, New York. p. 267–297.
- Iiyama, K. & Wallis, A.F.A. 1988. An improved acetyl bromide procedure for determining lignin in woods and wood pulps. *Wood Science and Technology* 22: 271–280.
- Jolliffe, I.T. 2002. *Principal component analysis*. 2nd ed. Springer-Verlag, New York. 487 p.
- KCL. 1982. *Massan ja puun kokonaisligniinipitoisuus* [Total lignin content of wood and pulp]. KCL, Espoo, Finland, 115b. 3 p. (In Finnish).
- Magel, E.A. 2000. Biochemistry and physiology of heartwood formation. In: Savidge R.A., Barnett, J.R. & Napier, R. (eds.). *Cell and molecular biology of wood formation*. BIOS Scientific Publishers Ltd, Oxford. p. 363–376.
- Mardia, K.V., Kent, J.T. & Bibby, J.M. 1979. *Multivariate analysis*. Academic Press, London. 521 p.
- Martens, H. & Næs, T. 1989. *Multivariate calibration*. John Wiley & Sons, Chichester. 419 p.
- Meder, R., Gallagher, S., Mackie, K.L., Böhler, H. & Meglen, R.R. 1999. Rapid determination of the chemical composition and density of *Pinus radiata* by PLS modelling of transmission and diffuse reflectance FTIR spectra. *Holzforschung* 53: 261–266.
- Næs, T., Isaksson, T., Fearn, T. & Davies, T. 2002. *A user-friendly guide to multivariate calibration and classification*. NIR publications, Chichester. 344 p.
- Panshin, A.J. & de Zeeuw, C. 1980. *Textbook of wood technology*. Structure, identification, properties,

- and uses of the commercial woods of the United States and Canada. 4th ed. McGraw-Hill, New York. 722 p.
- Raiskila, S., Fagerstedt, K., Laakso, T., Saranpää, P., Löjja, M., Paajanen, L., Mahlberg, R. & Ritschkoff, A.-C. 2006a. Polymerisation of added coniferyl alcohol by inherent xylem peroxidases and its effect on fungal decay resistance of Norway spruce. *Wood Science and Technology* 40(8): 697–707.
- , Saranpää, P., Fagerstedt, K., Laakso, T., Löjja, M., Mahlberg, R., Paajanen, L. & Ritschkoff, A.-C. 2006b. Growth rate and wood properties of Norway spruce cutting clones on different sites. *Silva Fennica* 40(2): 247–256.
- Rodrigues, J., Faix, O. & Pereira, H. 1998. Determination of lignin content of *Eucalyptus globulus* wood using FTIR spectroscopy. *Holzforschung* 52: 46–50.
- Saranpää, P. 2002. Ensiharvennuskruusen raakaineominaisuudet. In: Saranpää, P. & Verkasalo, E. (eds.). *Kruusen laatu ja arvo*. Finnish Forest Research Institute, Research Papers 822. p. 61–70. (In Finnish).
- Sjöström, E. 1993. *Wood chemistry – fundamentals and applications*. Academic Press, Inc., USA. 293 p.
- Venables, W.N. & Ripley, B.D. 1997. *Modern applied statistics with S-PLUS*. 2nd ed. Springer-Verlag, New York. 548 p.
- Wadenbäck, J., Clapham, D., Gellerstedt, G. & von Arnold, S. 2004. Variation in content and composition of lignin in young wood of Norway spruce. *Holzforschung* 58: 107–115.
- Walker, J.C.F. 1993. *Primary wood processing, principles and practice*. Chapman & Hall, London. 595 p.
- Weisberg, S. 1985. *Applied linear regression*. John Wiley & Sons, New York. 324 p.
- Westad, F. & Martens, H. 2000. Variable selection in near infrared spectroscopy based on significance testing in partial least squares regression. *Journal of Near Infrared Spectroscopy* 8(2): 117–124.

Total of 36 references