Chemometric Study on Alkaline Pre-treatments of Wood Chips Prior to Pulping

Joni T. Lehto,[a,]* Jarmo Louhelainen,[a] Hannu Pakkanen,[a] Petteri Malkavaara,[b]
Teresa Kłosińska,[c] Michał Drożdżek,[c] and Raimo Alén [a]

Alkaline pre-treatments were performed for the production of organics-containing effluents from silver/white birch (*Betula pendula/pubescens*) and Scots pine (*Pinus sylvestris*) chips prior to chemical pulping. Pre-treatment conditions were varied with respect to time (from 30 min to 120 min), temperature (130 °C and 150 °C), and alkali charge (1, 2, 3, 4, 6, and 8% of NaOH on oven-dried wood). The analytical data (total content, weight average molar mass, and molar mass distribution) on dissolved lignin were subjected to principal component analysis to examine the relationship between molar mass and molar mass distributions in lignin removed from different wood species under varying alkaline pre-treatment conditions. Using this method, differences between the wood species and effects of the various pre-treatment process variables (*i.e.,* time, temperature, and alkali charge) were determined.

Keywords: Alkaline pre-treatment; Biorefining; Chemical pulping; Chemometrics; Lignin; Molar mass distribution; Principal component analysis; Scots pine; Silver/white birch

Contact information: a: Department of Chemistry, University of Jyväskylä, FI-40014 Jyväskylä, Finland; b: Laboratory Service Unit, University of Tampere, FI-33014 Tampere, Finland; c: Faculty of Wood Technology, Warsaw University of Life Sciences-SGGW, 02-787 Warsaw, Poland;
** Corresponding author: joni.t.lehto@jyu.fi*

INTRODUCTION

Conventional chemical pulp mills can be seen as typical chemical thermochemical biorefineries that fractionate and convert woody biomass into a wide range of products, such as cellulose, lignin fragments, carbohydrate-based organic acids, and extractives-derived by-products (Kamm *et al.* 2006; Alén 2011; Lehto 2015). However, a more effective transformation of conventional pulp mills into integrated forest biorefineries (IFBRs) capable of producing new biomaterials and renewable energy besides chemical pulp has gained growing international interest (van Heiningen 2006; Baijpai 2012). The overall target of sophisticated IFBR concepts is to maximize the benefits and revenues of the chemical pulping process by manufacturing novel bioproducts from currently underutilized process side streams, while simultaneously meeting the growing demand for high quality pulp and paper products (Mansoornejad *et al*. 2013; Martin-Sampedro *et al.* 2014). In one attractive alternative, a chemical pre-extraction step of chips prior to chemical pulping is applied to the recovery of hemicelluloses-, lignin-, and extractives-derived components. These fractions are further fractionated, purified, and utilized for producing various chemicals and polymer applications (Carvalheiro *et al*. 2008; von Weymarn 2011). The reactivity of feedstock material is increased *via* various pre-treatments, leading to enhanced pulping performance and recovery of potential by-streams (lignin and hydroxy acids) from the final black liquor.

Several chemical pre-treatments have been suggested for recovering different organic components from lignocellulosic materials; the most common ones are pressurized hot-water extraction (PHWE), acidic hydrolysis, and alkaline pre-treatment (Lehto 2015; Lehto and Alén 2015). Of these alternatives, the alkaline extraction of wood chips is the most promising when combined with an existing industrial alkaline pulping process (Yoon *et al*. 2011; von Schenck *et al*. 2013), as it reduces the cooking times and the need for alkali charge in the subsequent pulping process. In addition, green and white pulping liquors can be used for pre-treatments (Walton *et al*. 2010; Yoon and van Heiningen 2010). However, as in chemical pulping, the reaction mechanisms of alkaline pre-treatments are extremely complex, unselective, and not fully understood. The dissolution of lignin includes very complex reaction paths and phenomena, which needs additional research, particularly if the utilization of lignin in biorefinery applications is considered. To be an economically and technically attractive process, the overall chemistry of pre-treatments should be known in detail.

Chemometrics is a scientific discipline utilizing mathematical and statistical methods to produce chemically relevant information from experimental data; this technique visualizes and displays data rather than reporting raw numerical values (Wold 1995; Wold and Sjöström 1998; Lehto *et al*. 2014). In particular, principal component analysis (PCA) is a very powerful tool for describing and visualizing chemical phenomena observed during the chemical pre-treatments of wood chips. In this study, the dissolution of lignin during alkaline pre-treatments was chemometrically investigated.

EXPERIMENTAL

Analysis of the Feedstock Materials

Industrial silver/white birch (*Betula pendula/pubescens*) and Scots pine (*Pinus sylvestris*) chips were obtained from the Metsä Fibre pulp mill (Äänekoski, Finland). The chips were laboratory-screened according to the SCAN-CM 40:94 standard (1994), and chips having knots, bark residues, and other visible impurities were manually removed. The maximum thickness of the selected chips was 7 mm, with a maximum width of 13 mm and a minimum width of 7 mm. For the chemical analyses, chips were ground in a Retsch SM100 cutting laboratory mill (Retsch GmbH, Haan, Germany) equipped with a bottom sieve with trapezoid holes (perforation size of < 1.0 mm) and stored in plastic bags. Prior to analysis, the moisture content of the samples was determined. All analyses were done with two parallel samples, and the results reported were calculated as percentages of the original dry wood.

The ground samples were extracted with acetone for 4 h (6 to 10 percolations per hour) in a Soxhlet apparatus, and the extractives content was determined according to TAPPI test method T280 pm-99 (1999). The extract was concentrated with a rotating vacuum evaporator (Heidolph VV2000, Gemini BV Laboratory, Apeldoorn, The Netherlands); drying was completed before weighing by means of a gentle nitrogen gas stream.

The lignin content of the extractives-free sawdust samples (each about 200 mg) was determined as the sum of acid-insoluble Klason lignin and acid-soluble lignin, in accordance with TAPPI standards T222 om-98 (1998), T249 cm-00 (2000), and T250 um-00 (2000). Sawdust was first treated with H_2SO_4, and the precipitated lignin was filtered off, washed, dried, and weighed. The content of acid-soluble lignin was determined using a Beckman DU 640 UV/Vis-spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA) at 205 nm after dilution of one portion of the hydrolysate with H_2SO_4 until the absorbance (A) was in the range of 0.3 to 0.8. The concentration of dissolved lignin (c, g/L) was calculated by Eq. 1,

$$c = \frac{A}{a*b}, \tag{1}$$

where a is absorptivity (110 L/(g•cm) for hardwoods and 120 L/(g•cm) for softwoods) (Swan 1965) and b is the light path (cm). The content of different monosaccharides (*i.e.*, arabinose, galactose, glucose, mannose, and xylose) in the Klason hydrolysates was determined as previously described (Lehto and Alén 2013).

Alkaline Pre-treatments

Laboratory-scale pre-treatment experiments of the screened birch and pine chips were conducted in 1.25-L rotating stainless steel autoclaves heated in an oil bath (CRS Autoclave System 420, CRS Reactor Engineering AB, Stenkullen, Sweden). Two treatment temperatures (130 °C and 150 °C) and four treatment times (30, 60, 90, and 120 min) were used, with a 30-min heating period added to the treatment time. The liquid-to-wood ratio was 5 L/kg. The chosen cooking liquors were aqueous NaOH adjusted to 1, 2, 3, 4, 6, and 8% NaOH (w/w) in oven-dried (o.d.) wood. After a sufficient total pre-treatment time, the pre-treated wood chips were separated from the effluents by filtration. Pre-treated chips were washed with tap water, and the yield was calculated based on the dry solids content of untreated and pre-treated chips. Filtered pre-treatment hydrolysates and wood chips were stored at -18 °C.

Analysis of Extracted Lignin

The dissolved lignin content in the pre-treatment effluents was determined using a Beckman DU 640 UV/Vis spectrophotometer at 280 nm. Samples were diluted with 0.1 M NaOH until the absorbance was in the range of 0.3 to 0.8. The absorptivity values used for softwood and hardwood lignins were 21.7 L/(g•cm) and 21.3 L/(g•cm), respectively (Alén and Hartus 1988).

The relative molar mass distributions (MMDs based on $\overline{M}_w$) of the dissolved lignin were determined from alkaline hydrolysates by size exclusion chromatography (SEC) using a Waters high performance liquid chromatography (HPLC) system (Millford, MA, USA) equipped with a column (460 mm × 10 mm inner diameter) filled with Superdex 75 gel (Pharmacia, Uppsala, Sweden) (Pakkanen and Alén 2012; Lehto *et al.* 2015). Samples were diluted with the eluent (0.1 M NaOH) before measurement and filtered with a nylon syringe filter (0.45 µm). The flow rate of the eluent (0.1 M NaOH filtered with a 0.45-µm membrane) was 0.3 mL/min at room temperature. Detection was performed using a Waters 996 photodiode array (PDA) detector within a wavelength range of 240 nm to 400 nm, and 280 nm was used for the determination of molar masses (MMs). Calibration of the GPC system was performed with a commercial set of protein standards (Sigma-Aldrich, St. Louis, USA) and a number of lignin-like monomer/oligomer model compounds including vanillin (Fluka Chemical Corporation, Buchs SG, Switzerland), dehydrodiacetovanillone (synthesized in the laboratory), rutin (Aldrich Chemical Company, Milwaukee, WI, USA), and tannic acid (Lancaster Synthesis, Morecambe, England) (Ristolainen *et al.* 1996).

Chemometric Analyses

The chemometric analysis of the experimental data obtained after the determination of the chemical compositions of the pre-treatment effluents was performed by using a personal computer equipped with Unscrambler® X v. 10.1 software (CAMO Software AS, Oslo, Norway).

PCA was used to visualize differences in the samples (and, therefore, the process) and to emphasize the contribution of each process variable to the notified differences. The main goal of the analysis was to establish a relationship between the applied alkaline pre-treatment conditions (*i.e.*, treatment time, temperature, and alkali charge) and the lignin dissolution pattern, while simultaneously presenting the differences between the two wood species utilized in the experimental set up.

RESULTS AND DISCUSSION

Feedstock Materials and Pre-treatments

Chemical compositions of the feedstock materials used in the alkaline pre-treatment experiments are presented in Table 1.

Table 1. Chemical Composition of Feedstock Materials Used in Alkaline Pre-treatment Experiments (% of the Dry Matter)

Component	Birch	Pine
Monosaccharides*	64.1	60.4
Arabinose	0.4	1.4
Galactose	1.3	1.8
Glucose	39.7	39.3
Mannose	1.6	13.8
Xylose	21.1	4.1
Lignin	22.1	27.7
Acid-soluble	4.8	0.5
Klason lignin	17.3	27.2
Extractives	3.7	3.1
Unidentified materials	8.2	10.7

*Monosaccharide moieties are presented as anhydro sugars.

The dry solid yields after the alkaline pre-treatments ranged between 97.9% and 83.5% for o.d. birch and between 98.0% and 86.4% for o.d. pine. High pre-treatment temperature, long treatment time, and high alkali charge clearly enhanced the formation of various hemicelluloses-derived carboxylic acids (volatile acids and hydroxy acids) corresponding to roughly 2/3 of the dissolved organic material. For this reason, extensive deacetylation of hemicelluloses (*i.e*., the release of acetic acid) as well as alkali-catalyzed peeling reactions leading to the formation of hydroxy acids under the pre-treatment conditions were evident. On the other hand, only very small contents of monosaccharides could be detected in the effluents.

Lignin Determinations

Slightly higher lignin concentrations (1.5 g/L to 9.5 g/L) were determined from pine effluents when compared to those (1.2 g/L to 6.9 g/L) from corresponding birch liquors. The mass loss in lignin corresponded to 0.8% to 4.4% of o.d. pine wood (2.9% to

15.9% of the total lignin) and 0.6% to 3.4% of o.d. birch wood (2.7% to 15.4% of the total lignin). Thus, increased alkali charge clearly enhanced the removal of lignin from both feedstocks. In addition, slightly higher relative weight average molar masses of the dissolved lignin were determined from pine effluents (from 2,260 g/mol to 7,050 g/mol), as the corresponding values for dissolved birch lignins varied from 2,200 g/mol to 5,550 g/mol.

MMDs of birch and pine lignins in effluents obtained under varying alkaline pre-treatment conditions are presented in Fig. 1. In general, harsh pre-treatment conditions enhanced the dissolution of large-MM lignin from the wood matrix. Clear differences in the lignin dissolution pattern were observed in the MMDs of birch and pine lignins. As was the case with weight average MMs, the MMD analysis revealed that lignin dissolved from pine chips had greater MMs than the corresponding birch lignin.

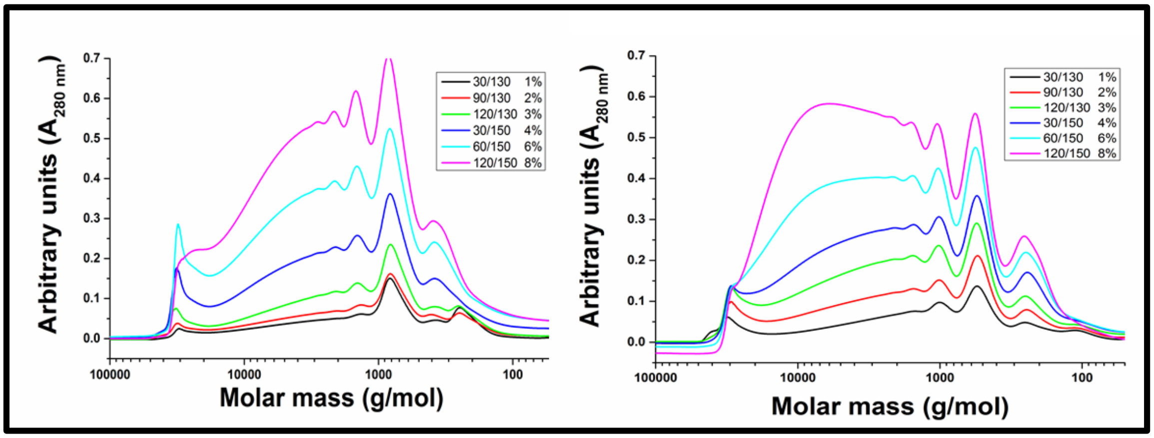

Fig. 1. MMDs of dissolved birch (left) and pine (right) lignin in alkaline effluents

Chemometric Analysis for the Lignin Dissolution

The PCA-score plot for lignin dissolution was calculated simultaneously for both wood species (Fig. 2).

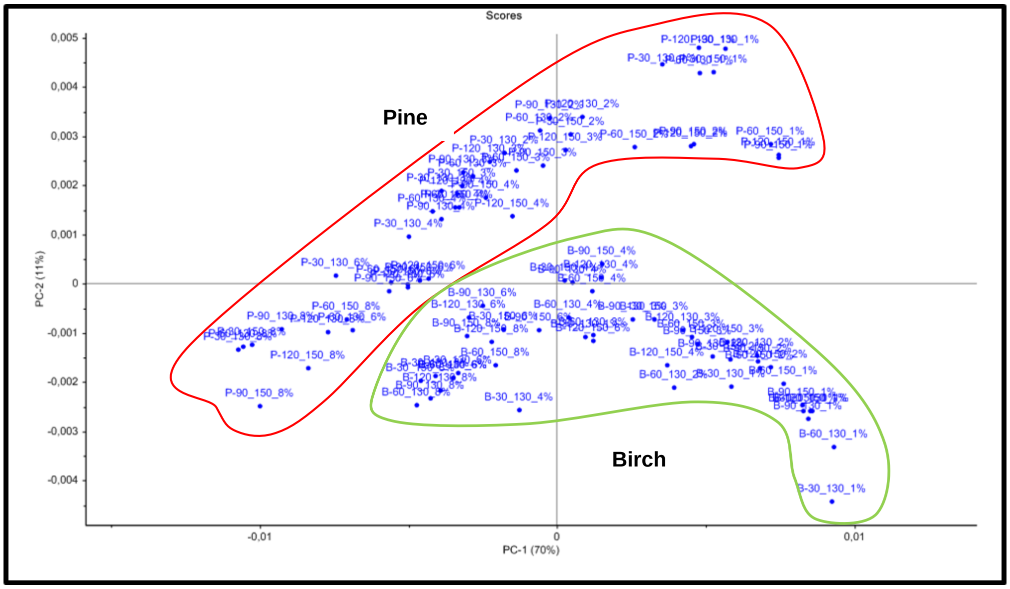

Fig. 2. PCA of the dissolved lignin for both wood species (P for pine and B for birch)

These calculations illustrated clear differences between the dissolution behavior of softwood and hardwood lignins. For this reason, individual PCAs were calculated separately for birch (Fig. 3) and pine (Fig. 4).

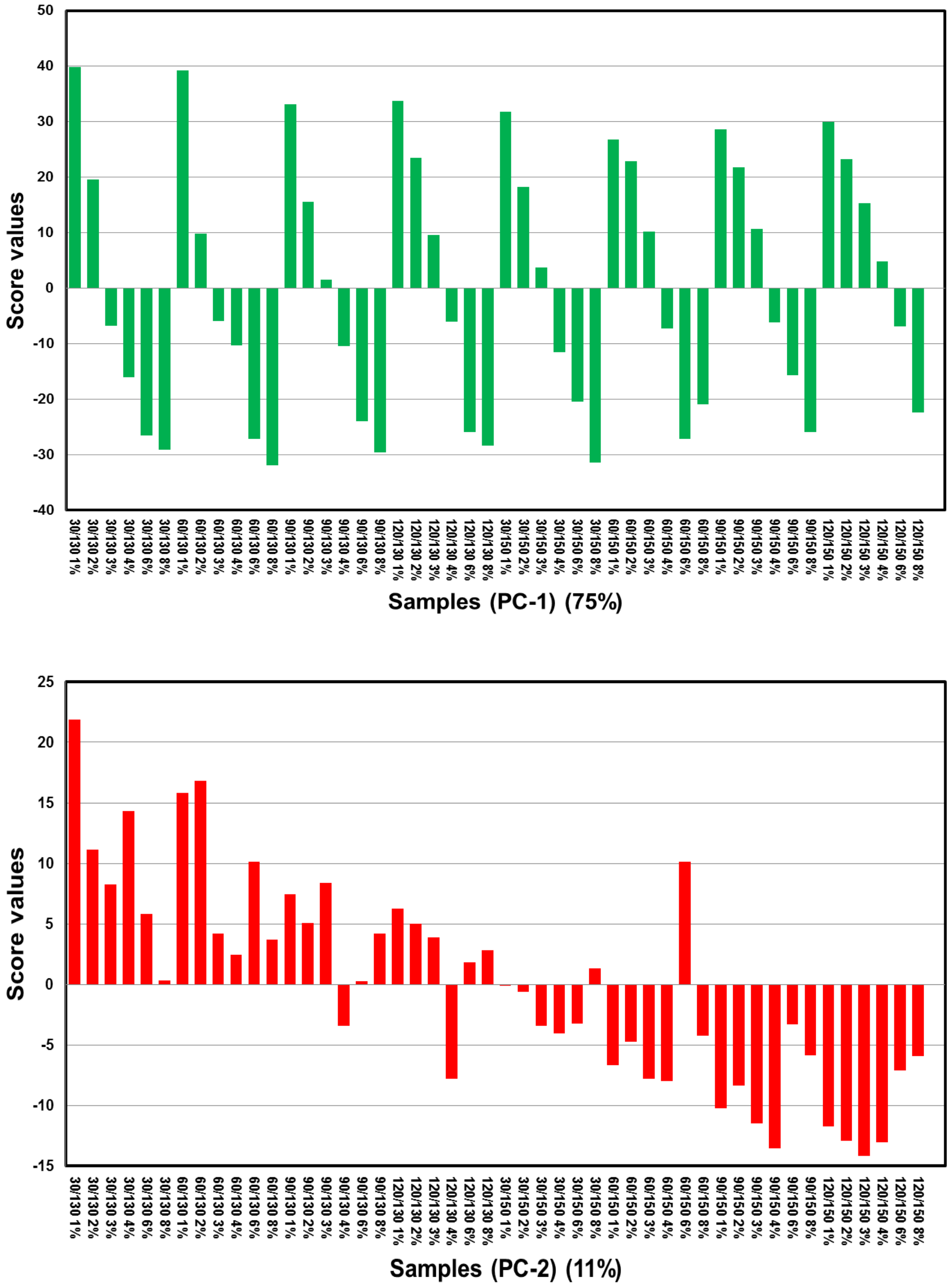

Fig. 3. First (top) and second (bottom) principal components (PCs) calculated for dissolved birch lignin

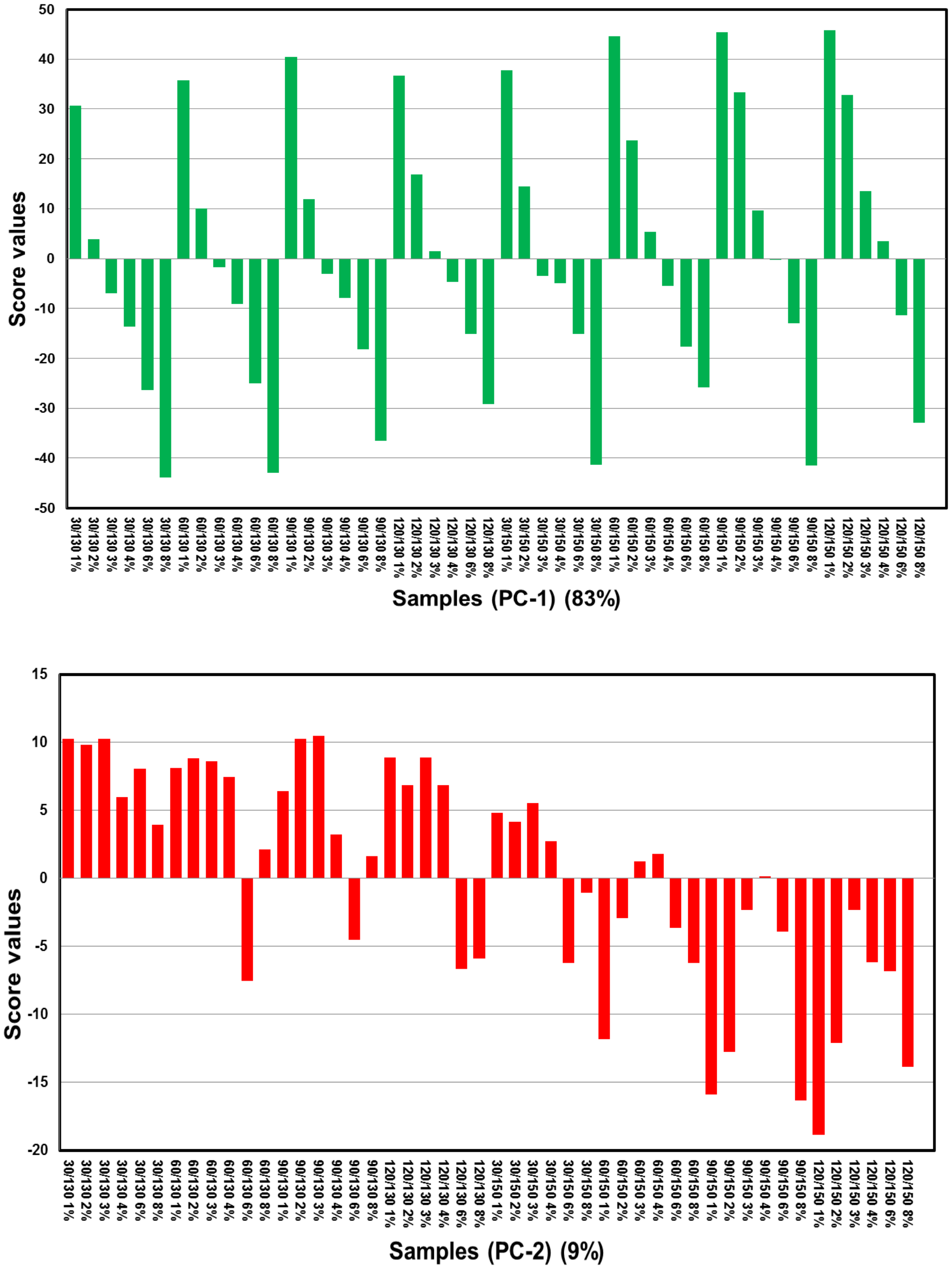

Fig. 4. First (top) and second (bottom) principal components (PCs) calculated for dissolved pine lignin

The corresponding loading chromatograms for the first two PCs and for both wood species are presented in Fig. 5. The PC calculations were used to pinpoint differences between the samples and to provide meaningful information from the analytical data. In this method, scores formed the projections of samples, and loading values were projections of the variables in the PC dimensions. The score values could then be used as samples and loading values as variables (in this case SEC chromatogram). In this method, each PC captured a portion of total variance in original data, and this portion was presented as a percentage in the figure captions. Value 75% for birch PC-1 signified that the first PC used 75% of the original data variance. By the nature of the PC calculations, the first PC captured the greatest amount of variance, the second PC captured the largest variance orthogonal to the first component, and the third PC captured the largest variance orthogonal to first and second, and so on. In the case of both wood species, the main process parameter (*i.e.*, PC-1) affecting the MMs and MMDs of the dissolved lignin was alkali charge. The effect of alkali charge was accountable for 75% (birch) and 83% (pine) of the variance considering the increasing MMs and MMDs of the dissolved lignin. The corresponding values for the second PCs (*i.e.*, PC-2) accounted for 11% and 9% of the variance for birch and pine, respectively. Compared with the applied alkali charge, increasing the treatment temperature and prolonging the treatment time did not have any significant effect on the lignin MMs or MMDs. The PCA-score plots of the first two PCs for birch and pine are illustrated in Figs. 6 and 7, respectively.

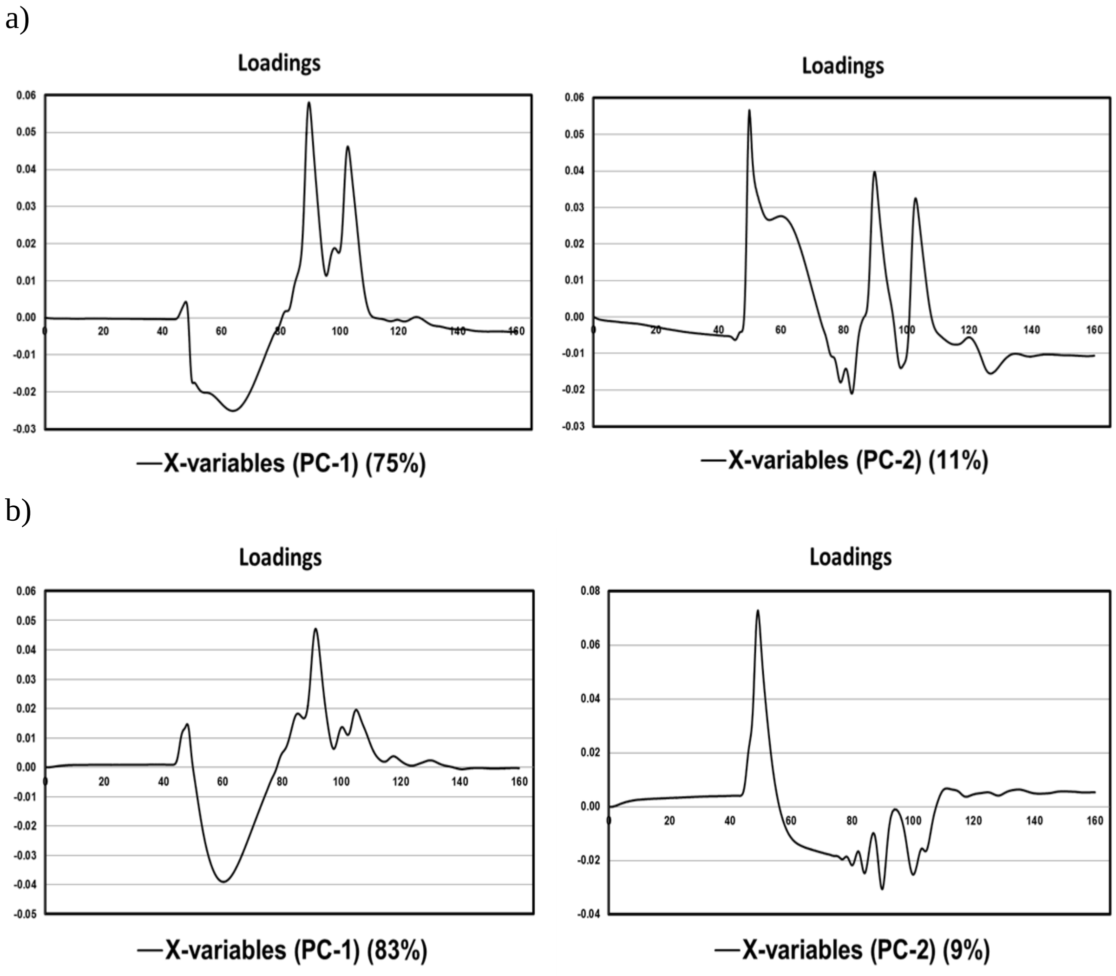

Fig. 5. Loading chromatograms of the first two PCs for birch (a) and pine (b)

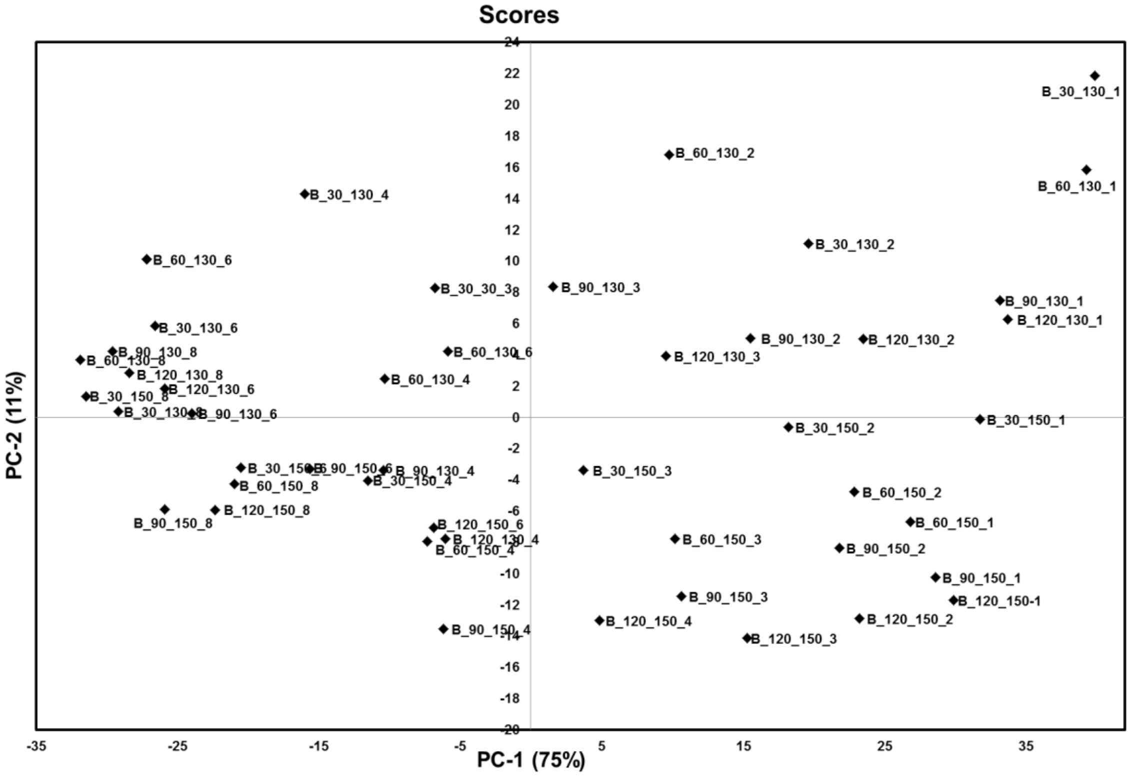

Fig. 6. PCA-score plot presenting first two PCs of birch lignin dissolution

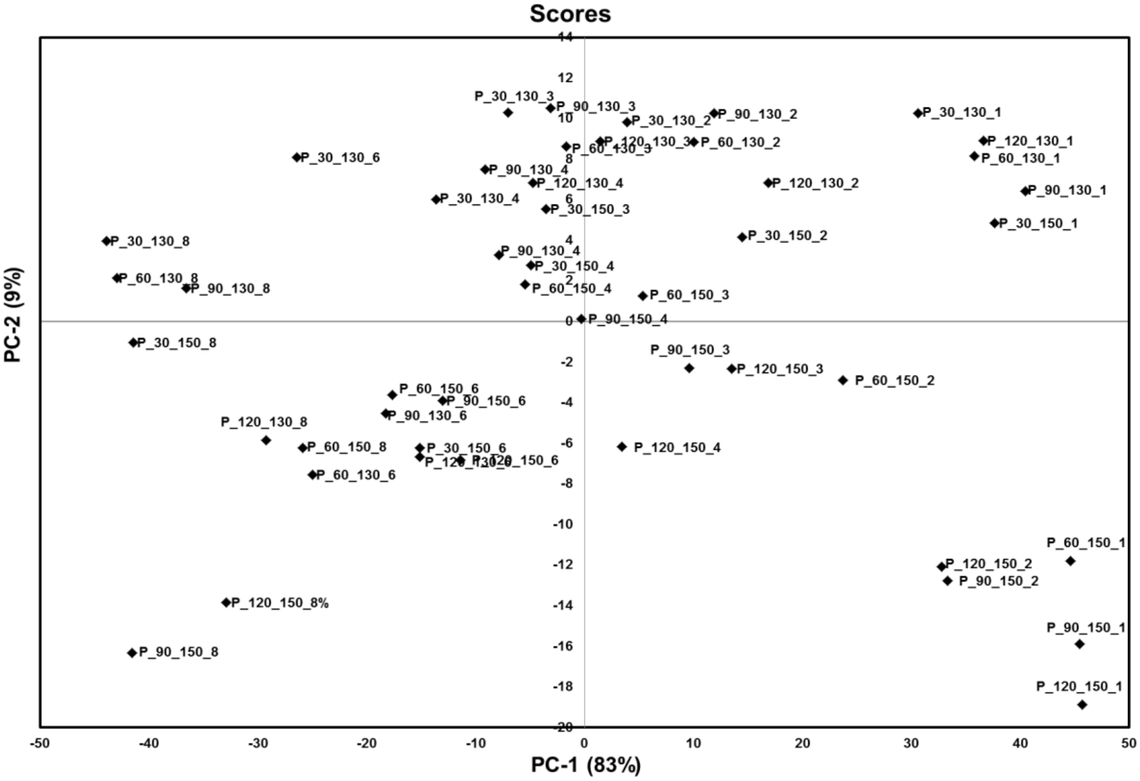

Fig. 7. PCA-score plot presenting first two PCs of pine lignin dissolution

The chemometric PC model was characterized both in terms of score values and loading values. In this manuscript, the score values represented the samples in principal coordinates, whereas the loading values could be interpreted similarly as the corresponding SEC chromatogram. The exception is that the higher the loading value (either positive or negative), the more influence the data point (*i.e.*, dissolved lignin with specific MMs) had in the PC in that dataset. Therefore, the loading chromatograms could be interpreted as a tool combining the information presented in the SEC chromatogram to the specific samples in the dataset. Based on the data presented in Fig. 5, the main difference between pine samples present in the second PC was attributed to the area of sharp main peak present at time point of 50 min (*i.e.,* the exclusion peak in the SEC chromatogram). In case for birch samples, the difference depicted as second PC was attributed into several areas, namely the time points of 50 min to 60 min (exclusion peak), 90 min to 95 min (MM area from 500 g/mol to 1000 g/mol), and around 105 min (MM area from 300 g/mol to 400 g/mol). In other words, second PC of pine dataset consisted of information regarding mainly higher-MM material compared to the birch dataset.

CONCLUSIONS

1. Alkaline pre-treatments conducted under various treatment conditions (*i.e*., 130 °C and 150 °C for 30, 60, 90, and 120 min) with different alkali charges (1, 2, 3, 4, 6, and 8% NaOH in o.d. wood) were effective in dissolving hemicelluloses- and lignin-derived components from common pulp woods (silver/white birch and Scots pine). The yields varied between 97.9% and 83.5% and between 98.0% and 86.4% for o.d. birch and pine, respectively.
2. The dissolved fraction was mainly composed of various hemicelluloses-derived carbohydrates and their degradation products, as well as lignin, and extractives. Increasing the alkali charge clearly facilitated the alkali-catalyzed degradation reactions of polysaccharides to various hydroxy acids. Under the most drastic pre-treatment conditions, 2/3 of the total dissolved organic fraction was composed of various aliphatic carboxylic acids, the residual material being mainly degraded lignin and extractives. In each case, only relatively small quantity of monosaccharides and trace amounts of furans could be analyzed.
3. Analysis of the content, MMs, and MMDs of dissolved lignin (2.9% to 15.9% of the total pine lignin and 2.7% to 15.4% of the total birch lignin) revealed distinct differences between the different wood species. These observations were strengthened by PCA, which suggested different dissolution behavior for softwood and hardwood lignins.
4. A basic chemometric technique PCA was shown to be a powerful tool for explaining chemical phenomena during the alkaline pre-treatment of two common industrial pulp woods. PCA verified that alkali charge had the most influence on MMDs and MMs (seen as increasing lignin MM), because 83% and 75% of the variance in lignin dissolution of pine and birch, respectively, was explained by this variable alone.
5. In general, an increase in the alkali charge seemed to enhance the solubility of lignin by dissolving more and higher-MM lignin from the wood. On the other hand, raising the pre-treatment temperature and prolonging the pre-treatment time seemed to lower

the $\overline{M}_w$ values, suggesting a further degradation of the dissolved lignin as the pre-treatment conditions were more severe.

ACKNOWLEDGEMENTS

The Metsä Fibre Äänekoski pulp mill is gratefully acknowledged for providing wood materials for the experimental work. Financial support from the Maj and Tor Nessling Foundation (Joni Lehto) and European Union in the context of the goals of "Europe 2020 Strategy" and in the framework of "Human Capital Operational Program No. POKL.04.0300-00-042/12-00" (Teresa Kłosińska and Michał Drożdżek) are gratefully acknowledged.

REFERENCES CITED

Alén, R. (2011). "Principles of biorefining," in: *Biorefining of Forest Resources*, Alén, R. (ed.), Paper Engineers' Association, Helsinki, Finland, pp. 18-114.

Alén, R., and Hartus, T. (1988) "UV spectrophotometric determination of lignin from alkaline pulping liquors," *Cell. Chem. Technol.* 22, 613-618.

Baijpai, P. (2012). "Integrated forest biorefinery," in: *Biotechnology for Pulp and Paper Processing*, Bajpai, P. (ed.), Springer, New York, NY, USA, pp. 375-402.

Carvalheiro, F., Duarte, L. C., and Gírio, F. M. (2008). "Hemicellulose biorefineries: A review on biomass pretreatments," *J. Sci. Ind. Res.* 67, 849-864.

Kamm, B., Kamm, M., Gruber, P. R., and Kromus, S. (2006). "Biorefinery systems – An overview," in: *Biorefineries–Industrial Processes and Products, Status Quo and Future Directions*, B. Kamm, P. R. Gruber, and M. Kamm (eds.), Wiley-Verlag, Weinheim, Germany, pp. 3-40.

Lehto, J. (2015). *Advanced Biorefinery Concepts Integrated to Chemical Pulping*, Ph.D. Dissertation, University of Jyväskylä, Jyväskylä, Finland.

Lehto, J., and Alén, R. (2013). "Alkaline pre-treatment of hardwood chips prior to delignification," *J. Wood Chem. Technol.* 33(2), 77-91. DOI: 10.1080/02773813.2012.748077

Lehto, J. T., and Alén, R. J. (2015). "Chemical pretreatments of wood chips prior to alkaline pulping - A review of pretreatment alternatives, chemical aspects of the resulting liquors, and pulping outcomes," *BioResources* 10(4), 8604-8656. DOI: 10.15376/biores.10.4.Lehto

Lehto, J., Alén, R., and Malkavaara, P. (2014). "Multivariate correlation between analytical data for various organics dissolved during autohydrolysis of silver birch (*Betula pendula*) chips and treatment conditions)," *BioResources* 9(3), 4958-4970. DOI: 10.1515/hf-2014-0094

Lehto, J., Pakkanen, H., and Alén, R. (2015). "Characterization of lignin dissolved during alkaline pre-treatment of softwood and hardwood," *J. Wood Chem. Technol*. 35(5), 337-347. DOI: 10.1080/02773813.2014.965332

Mansoornejad, B., Pistikopoulos, E. N., and Stuart, P. (2013). "Metrics for evaluating the forest biorefinery supply chain performance," *Comp. Chem. Eng.* 57, 125-139. DOI: 10.1016/j.compchemeng.2013.03.031

Martin-Sampedro, R., Eugenio, M. E., Moreno, J. A., Revilla, E., and Villar, J. C. (2014). "Integration of a kraft pulping mill into a forest biorefinery: Pre-extraction of hemicellulose by steam explosion versus steam treatment," *Bioresour. Technol.* 153, 236-244. DOI: 10.1016/j.biortech.2013.11.088

Pakkanen, H., and Alén, R. (2012). "Molecular mass distribution of lignin from the alkaline pulping of hardwood, softwood, and wheat straw," *J. Wood Chem. Technol*. 32(4), 279-293. DOI: 10.1080/02773813.2012.659321

Ristolainen, M., Alén, R., and Knuutinen, J. (1996). "Characterization of TCF effluents from kraft pulp bleaching. I. Fractionation of hardwood lignin-derived material by GPC and UF," *Holzforschung* 50(1), 91-96. DOI: 10.1515/hfsg.1996.50.1.91

SCAN-CM 40:94. (1994). "Wood chips for pulp production - Size distribution," Scandinavian Pulp, Paper and Board Testing Committee, Stockholm, Sweden.

Swan, B. (1965). "Isolation of acid-soluble lignin from the Klason lignin determination," *Svensk Papperstidn*. 68(22), 791-795.

TAPPI T222 om-98. (1998). "Acid insoluble lignin in wood and pulp," TAPPI Press, Atlanta, GA, USA.

TAPPI T280 pm-99. (1999). "Acetone extractives of wood and pulp," TAPPI Press, Atlanta, GA, USA.

TAPPI T249 cm-00. (2000). "Carbohydrate composition of extractive-free wood and wood pulp by gas-liquid chromatography," TAPPI Press, Atlanta, GA, USA.

TAPPI T250 um-00. (2000). "Acid-soluble lignin in wood and pulp," TAPPI Press, Atlanta, GA, USA.

van Heiningen, A. (2006). "Converting a kraft pulp mill into an integrated forest biorefinery," *Pulp Paper Can.* 107(6), 38-43.

von Schenck, A., Berlin, N., and Uusitalo, J. (2013). "Ethanol from Nordic wood raw material by simplified alkaline soda cooking pre-treatment," *Appl. En*ergy 102, 229-240. DOI: 10.1016/j.apenergy.2012.10.003

von Weymarn, N. (2011). "The sugar platform in the wood biorefinery," in: *Nordic Wood and Biorefinery Conference,* Stockholm, Sweden, p. 138.

Walton, S. L., Hutto, D., Genco, J. M., van Walsum, G. P., and van Heiningen, A. R. P. (2010). "Pre-extraction of hemicelluloses from hardwood chips using an alkaline wood pulping solution followed by kraft pulping of the extracted wood chips," *Ind. Eng. Chem. Res.* 49(24), 12638-12645. DOI: 10.1021/ie100848p

Wold, S. (1995). "Chemometrics: What do we mean with it, and what do we want from it?" *Chemom. Intell. Lab. Syst.* 30(1), 109-115. DOI: 10.1016/0169-7439(95)00042-9

Wold, S., and Sjöström, M. (1998). "Chemometrics, present and future success," *Chemom. Intell. Lab. Syst.* 44(1-2), 3-14. DOI: 10.1016/S0169-7439(98)00075-6

Yoon, S.-H., and van Heiningen, A. (2010). "Green liquor extraction of hemicelluloses from southern pine in an Integrated Forest Biorefinery," *J. Ind. Eng. Chem.* 16(1), 74-80. DOI: 10.1016/j.jiec.2010.01.018

Yoon, S.-H., Tunc, M. S., and van Heiningen, A. (2011). "Near-neutral pre-extraction of hemicelluloses and subsequent kraft pulping of southern mixed hardwoods," *TAPPI J.* 10(1), 7-15.

Article submitted: December 30, 2015; Peer review completed: February 19, 2016; Revised version received and accepted: March 29, 2016; Published: April 6, 2016.
DOI: 10.15376/biores.11.2.4621-4632