Multivariate Correlation between Analytical Data for Various Organics Dissolved during Autohydrolysis of Silver Birch (*Betula pendula*) Chips and Treatment Conditions

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Autohydrolysis pre-treatments were performed for the production of hemicellulose-rich autohydrolysates from silver birch (*Betula pendula*) chips prior to chemical pulping. Pre-treatment conditions were varied with respect to time (from 30 to 120 min) and temperature (130 and 150 °C), covering a P-factor range from 10 to 238. Hydrolysates were analyzed in terms of carbohydrates, lignin, volatile organic acids, and furanoic compounds. The analytical data were subjected to various chemometric techniques to establish the relationships between dissolved organic components, hardwood and softwood used in the experiments, and applied pre-treatment conditions. Using this method, differences between the wood species could be clearly seen, and a relatively accurate model for the autohydrolysis of birch chips was developed.

Keywords: Autohydrolysis; Betula pendula; Carbohydrates; Volatile acids; Lignin; Furans; Biorefining; Principal component analysis

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INTRODUCTION

Biorefining generally refers to a versatile process in which biomass is fractionated into different product streams such as fuel, power, and value-added chemicals (Kamm *et al.* 2006; Carvalheiro *et al.* 2008; Amidon and Liu 2009; Alén 2011). With forest biomass, biorefining mainly involves the fractionation and conversion of the chemical components of wood (cellulose, hemicelluloses, and lignin) into desired products (Kamm and Kamm 2004; Alén 2011). Within this context, acidic pre-treatment processes, such as pressurized hot-water treatment (PHWT)—also known as autohydrolysis—integrated with existing pulp mills, are considered to be the most promising methods for the production of carbohydrate-rich hydrolysates, which can then be further processed into a wide range of useful chemicals (Garrote *et al.* 1999; Tunc and van Heiningen 2008; Kumar *et al.* 2009; Alvira *et al.* 2010; Girío *et al.* 2010; Teo *et al.* 2010).

The clear advantage of autohydrolysis is that water is the only reagent used, and additional chemicals are not needed. During autohydrolysis, hydronium-catalyzed reactions degrade and dissolve wood components into aqueous media (Testova *et al.* 2009; Borrega *et al.* 2011). At the first stage of the overall treatment, hydronium ions are generated through the autoionization of water molecules. As the reaction proceeds, acetyl groups present in hemicelluloses are cleaved by the effect of hydronium ions with the simultaneous formation of acetic acid and gradually followed by the partial degradation of

glycosidic bonds between monosaccharide moieties. As a result, under these acidic conditions, hemicellulose fragments (*i.e.*, mono-, oligo-, and polysaccharides) in particular, as well as their conversion products (*i.e.*, furanoic compounds 2-furaldehyde or furfural (FF), and 5-(hydroxymethyl) furfural (HMF)), are dissolved and incorporated into aqueous media (Tunc and van Heiningen 2011). In addition to these carbohydrate-derived components, the process generates a great number of organic degradation products of lignin and extractives.

Chemometrics is a scientific discipline that utilizes mathematical and statistical methods for extracting chemically relevant information from data produced in chemical experiments (Wold 1995). Chemometrics includes powerful tools for visualizing, representing, and displaying chemical data, instead of just representing raw numerical values (Wold and Sjöström 1998). Several chemometric techniques have been successfully used to describe various wood fractionation processes, especially in the interpretation of spectroscopic data (Schultz *et al.* 1985; Schultz and Burns 1990; Hyötyläinen *et al.* 1998; Malkavaara and Alén 1998; Malkavaara *et al.* 2000). Wood processing chemistry utilizes various feedstock materials, fractionation processes, and several analytical techniques adopted from analytical organic and inorganic chemistry. Various chemometric techniques (such as multivariate analysis) show many promising features for describing and monitoring these sophisticated processes and ensuring the product quality. In our previous study, we used well-known chemometric techniques to develop a relatively accurate model of the analysis data for dissolved organic material from Scots pine (*Pinus sylvestris*) chips and their autohydrolysis pre-treatment conditions (Lehto *et al.* 2014). However, preliminary results also indicated a profound disparity between softwood (Scots pine) and hardwood (silver birch) in the autohydrolysis experiments. As both of these wood species are extensively utilized by Nordic pulp companies and due to their clearly different behavior during autohydrolysis, it is highly beneficial to gather all information available about the analytical data concerning the autohydrolysis of softwood and hardwood chips prior to delignification. These facts together suggested the need for separate chemometric models for both wood species.

The main aim of the current study was to investigate various phenomena that occur in the autohydrolysis of silver birch (*Betula pendula*) chips and, using a chemometric approach, to establish correlations between the organic materials removed and the treatment conditions applied. In addition, we briefly explored the differences between the autohydrolysis behaviors of birch and pine.

EXPERIMENTAL

Autohydrolysis Procedures

Silver/white birch (*Betula pendula/pubescens*) wood chips of industrial origin were screened and pre-treated by autohydrolysis in a laboratory-scale oil-heated rotating batch digester (1.25 L, CRS Autoclave System 420). Knots, bark, and other visible impurities were manually removed from the samples before pre-treatments. Screened chips (max. thickness 7 mm, max. width 13 mm, and min. width 7 mm) were heated with ultra-high quality (UHQ) water (internal resistance ≥ 18.2 MΩcm at 25 °C) obtained from a Milli-Q Plus water system (Millipore, Bedford) to two maximum temperatures (130 and 150 °C). The chips were treated for four treatment times (30, 60, 90, and 120 min) and a 30 minutes heating-up time was added to these treatment times. The combined overall effect of

autohydrolysis time and temperature was represented by a single numerical value, the "P-factor" (pre-hydrolysis-factor), which is analogous to the "H-factor" used for similar purposes in chemical pulping (Sixta 2006; Tunc and van Heiningen 2009). Under applied pre-treatment conditions, the pre-treatments covered a P-factor range from 10 to 238. The liquid-to-wood ratio was 5 L/kg. After the sufficient total treatment time, the autoclaves were cooled in cold water. The hydrolysates were separated from the pre-treated chips by filtration bags and stored in a freezer for further analyses.

Analytical Determinations

The pre-treatment hydrolysates were characterized in terms of dissolved solids (DS) and concentrations of individual component groups including carbohydrates, lignin, volatile organic acids, and furanoic compounds.

Total contents of the carbohydrates and uronic acids were determined by an Agilent 6890 Series gas chromatography (GC) system after acid methanolysis using the methods developed earlier (Sundberg *et al.* 1996; Bertaud *et al.* 2002). Sorbitol (100 mg/L in methanol) was used as an internal standard (ISTD). Standard solution (100 mg/L in UHQ water) was prepared from arabinose, galactose, glucose, xylose, mannose, galacturonic acid, and glucuronic acid. In addition, for the identification of the monosaccharide peaks from the chromatogram, individual samples (0.1 mg/mL) were prepared from each monosaccharide. Samples were prepared by applying 1 mL of 1:10 diluted hydrolysate into a 10-mL pear shape flask and by drying the sample to dryness with a rotary evaporator. After drying, 2 mL of a dry methanolysis reagent (16 mL of acetyl chloride in 100 mL of methanol) was added to the flasks. Samples were mixed in an ultrasonic bath. Sample flasks were sealed tightly, and kept in the oven at 100 °C for 3 h. The flasks were let to cool down before opening. After cooling down, 80 µL of dried pyridine was added to the flasks to neutralize the possible hydrochloric acid present in the samples. After neutralization, 1 mL of ISTD was added to the flasks and the samples were dried with a rotary evaporator. Dried samples were stored in a freezer. The sample derivatization was started by diluting the carbohydrate components with 1 mL of dry pyridine (dried with KOH pellets), and silylated derivatives were prepared by adding 250 µL of silylating reagent (99 % BSTFA + 1 % TMCS) to the samples, mixing the samples in an ultrasound bath for a few minutes, and shaking the samples for 40 minutes to ensure the proper derivatization. The samples were filtrated, transferred to a glass vials, and analyzed with a GC. GC system was equipped with an HP-5 column (length, 30 m, inner diameter 0.32 mm, and film thickness 0.25 µm), and a flame ionization detector (FID).

The total content of soluble lignin (TL) in hydrolysates was determined with a Beckman DU 640 UV/Vis-spectrophotometer operated at 205 nm. The concentration of dissolved lignin (c, g/L) was calculated according to the equation,

$$c = \frac{A}{a*b}, \quad (1)$$

where a is absorptivity value for dissolved birch lignin (110 L/(gcm) (Swan 1965)) and b is light path (cm) (Lehto and Alén 2012).

Volatile carboxylic acids (*i.e.*, acetic and formic acids) were determined by means of the previously described method (Käkölä *et al.* 2008) using a Dionex HPLC chromatography system equipped with an AS50 autosampler, a LC25 chromatography oven, an EG40 eluent (KOH/UHQ water) generator, and an IC25 ion chromatograph. The

system was equipped with an anion trap column (IonPac ATC-1), and the separation column was an IonPac AS 11-HC analytical column combined with an AG11-HC guard column (Dionex). Samples were injected via a 25 µL-loop and were eluted (KOH/UHQ water) at a flow rate of 1.0 mL/min. Data were stored and processed using a Dionex Chromeleon (6.50) data system. The identification of the chromatographic peaks was based on the model substances sodium acetate (J.T. Baker) and sodium formiate (Riedel-de Haën).

Furanoic compounds (acidic degradation products originated from carbohydrates, *i.e*., FF and HMF) (TF) were determined using an HPLC equipment containing 510 pumps (Waters), a 717 injection system, a 996 diode-array detector (DAD), and a Phenomenex Gemini C18 column. The injection volume was 30 µL and the detection wavelength was 280 nm (UV-region). UHQ-water/acetonitrile (ACN from J.T. Baker) mixture (in a volume ratio of 9:1) and pure ACN were used as eluents. The eluent flow rate was 1.0 mL/min. The identification and quantitation of the chromatographic peaks was based on the standard samples prepared from model substances FF (from Aldrich) and HMF (from Aldrich) (Lehto and Alén 2012).

Data Analysis

The data analysis and pre-treatment procedures were performed in a similar manner as described earlier (Lehto *et al.* 2014). The data analysis methods used were the principal component analysis (PCA) and projection to latent structures (PLS) regression. All computations were carried out on a personal computer using the Unscrambler® X software package (Unscrambler® 2011).

Principal component analysis was used mainly to visualize the differences amongst the studied samples (and therefore, the studied process), and to emphasize the contribution of each variable to the notified differences. The main aspect of using PLS regression method was to find and study multivariate relationships between the analytical results and interesting process variables.

RESULTS AND DISCUSSION

Principal Component Analysis

In the previous study (Lehto *et al.* 2014), the PCA model based on the analytical data for softwood and hardwood revealed clear differences between the samples, and two distinct groups were found, one for each wood species, suggesting clearly their different behavior during autohydrolysis. The differences were clear with respect to the P-factor and wood species—TC, DS, and P-factor being the most influential variables in the sample grouping. In this study, Table 1 presents the detailed analytical data for birch samples, and Figs. 1 and 2 show the PCA model prepared for birch samples from these data.

As expected, the chemical composition of the hardwood hydrolysates was clearly different from that of the corresponding softwood hydrolysates. In general, less organic material was dissolved from the birch chips during pre-treatments performed under low temperatures with short pre-treatment times (*i.e*., low P-factors). In contrast, longer pre-treatment times combined with higher pre-treatment temperatures (*i.e*., high P-factors) clearly enhanced the dissolution rate of organics. This difference was seen especially with TL and TA, as the content of TL was ~2.5 times higher and that of TA ~3.5 times higher

in birch hydrolysates than in pine hydrolysates pre-treated under the same conditions (*i.e.*, with a P-factor of 238).

Table 1. Analytical Birch Hydrolysate Data Subjected to PCA Calculations

With the exception of P-factor, all the other values are expressed as (g *10^{-3})/g						
Symbol*	P-factor	TC	TA	TL	TF	DS
B1	10	4.95	1.91	5.79	0.01	13.95
B2	20	8.29	2.39	7.50	0.03	18.68
B3	30	9.36	2.73	9.46	0.03	21.35
B4	41	13.89	3.66	11.17	0.05	28.78
B5	60	30.31	4.97	13.59	0.12	45.95
B6	119	73.09	12.25	19.49	0.35	97.50
B7	179	126.85	16.39	21.31	0.43	150.80
B8	238	147.18	17.94	24.46	1.25	172.60
*TC refers to the total amount of carbohydrates, TA to the total amount of volatile acids, TL to the total amount of lignin, TF to the total amount of furans, and DS to the total amount of dissolved solids.						

The content of TA can be explained by the chemical composition of hardwood xylan, which is highly acetylated; the degree of acetylation varies between 8 to 15 % of the total xylan, corresponding 3.5 to 7 acetyl groups per 10 xylose units (Alén 2011). On the other hand, softwood xylan is not acetylated and glucomannan is only partly acetylated (~6 % acetyl group content). As the formation of TA is essential for the autohydrolysis process, this kind of difference between the two studied wood species is significant when regarding the efficiency of the autohydrolysis.

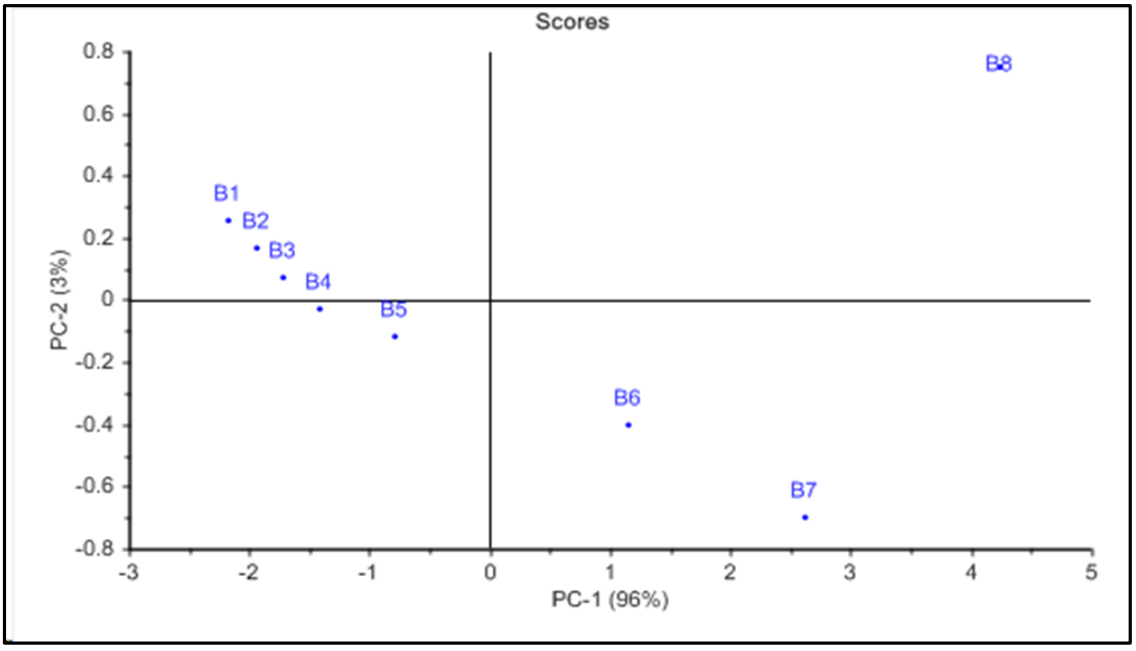

Fig. 1. PCA score plot of birch samples B1 through B8 using the first and second principal components (PCs). For samples, see Table 1.

The calculated PCA model for the birch samples revealed that there were two separate phases in the autohydrolysis process, detected as groupings of the score values of samples B1 – B5, and B6 - B8. The heterogeneity within each group was clearly increased with the increasing P-factor, mostly because of TF and TL, detected as loading values of the variables TF and TL. This observation was consistent with the previous study conducted with pine samples (Lehto *et al.* 2014).

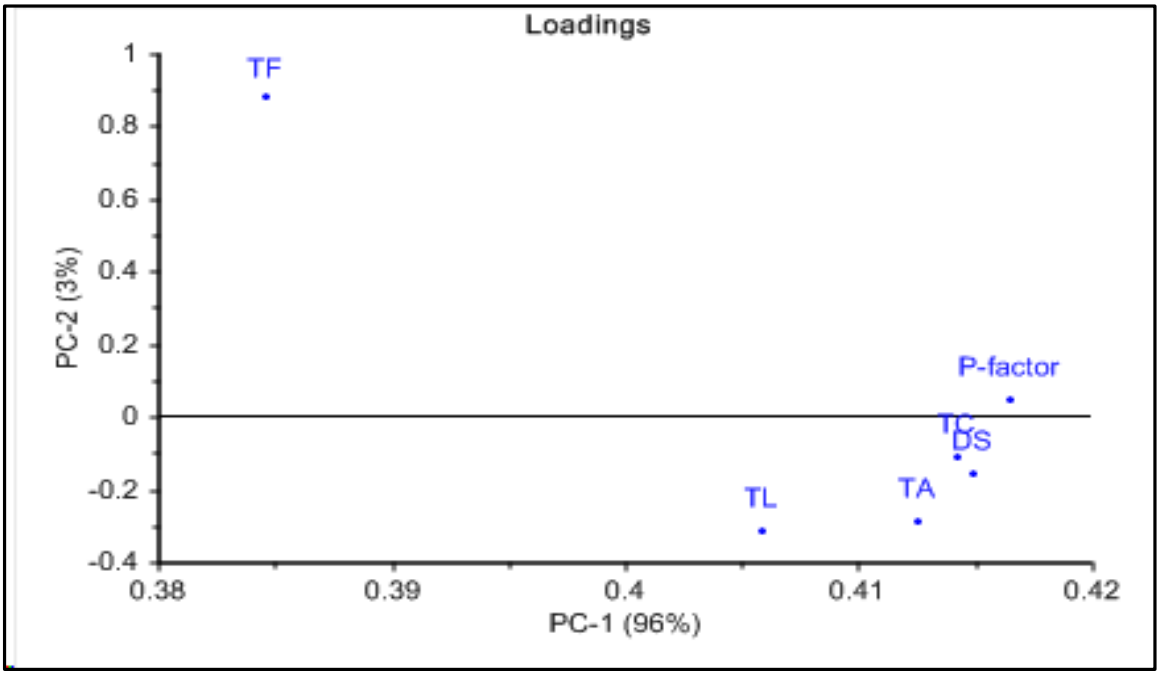

Fig. 2. Loading plot for the PCA model with birch samples using the first and second PCs. For abbreviations, see Table 1.

In general, when comparing the data in Table 1 and Fig. 2, a clear connection can be observed between the increasing P-factor and the dissolved organic materials (especially the different carbohydrates, their degradation products, and the furanoic compounds). The effect of these parameters on the PCA model was also clear. Because the concentration of carbohydrates remained continuously high during the autohydrolysis process, their influence was the most significant. On the other hand, because furanoic compounds formed from carbohydrates only after prolonged pre-treatment times, they only affected the PCA model at high P-factors.

Table 2 presents the coefficients of determination (R^2) for the first and second principal components of both PCA models. These coefficients showed no significant differences when compared with the PCA models constructed using pine samples (Lehto *et al.* 2014).

Table 2. Coefficients of Determination (R^2, in Cumulative %) for the PCA Models

Rank	PCA with pine and birch samples	PCA with birch samples
1	86	96
2	98	99

Projection to Latent Structures Regression

The PLS model for the P-factor and another PLS model for the DS were calculated from the analytical data of birch samples and cross-validated using the full cross-validation procedure (Wold *et al.* 2001). The cross-validation results for these PLS models are presented in Table 3. The predicted values for P-factor and DS, calculated using the cross-validated PLS models, are plotted against the analytically determined values in the Figs. 3 and 4, respectively.

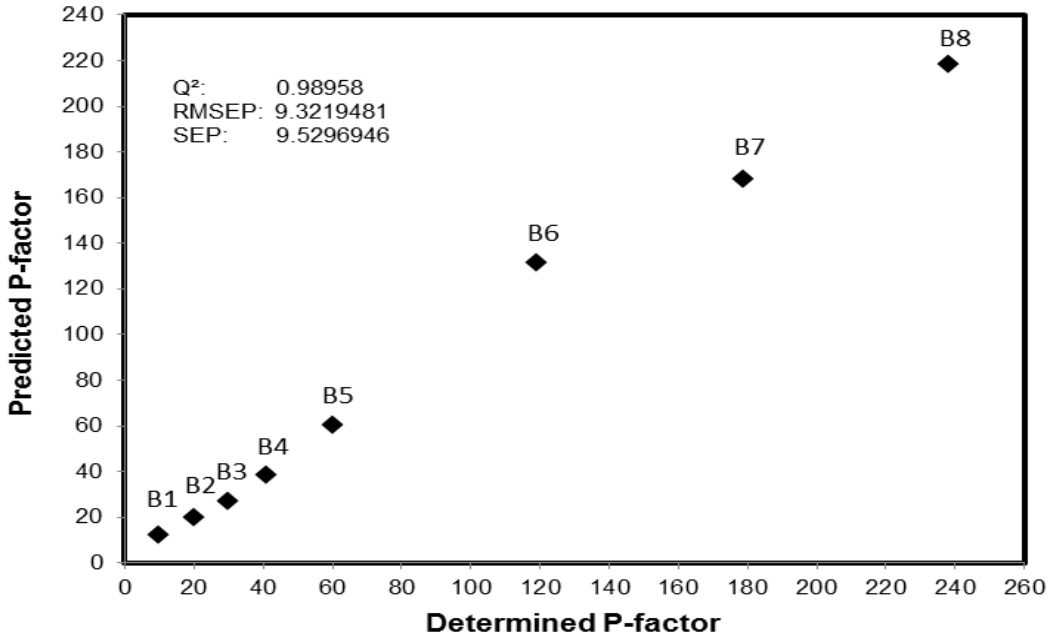

Fig. 3. Predicted *vs*. determined P-factor values for the birch samples (see Table 1). Q^2 is the cross-validated coefficient of determination (in cumulative %), RMSEP the root mean square error, and SEP the standard error of prediction (in original units).

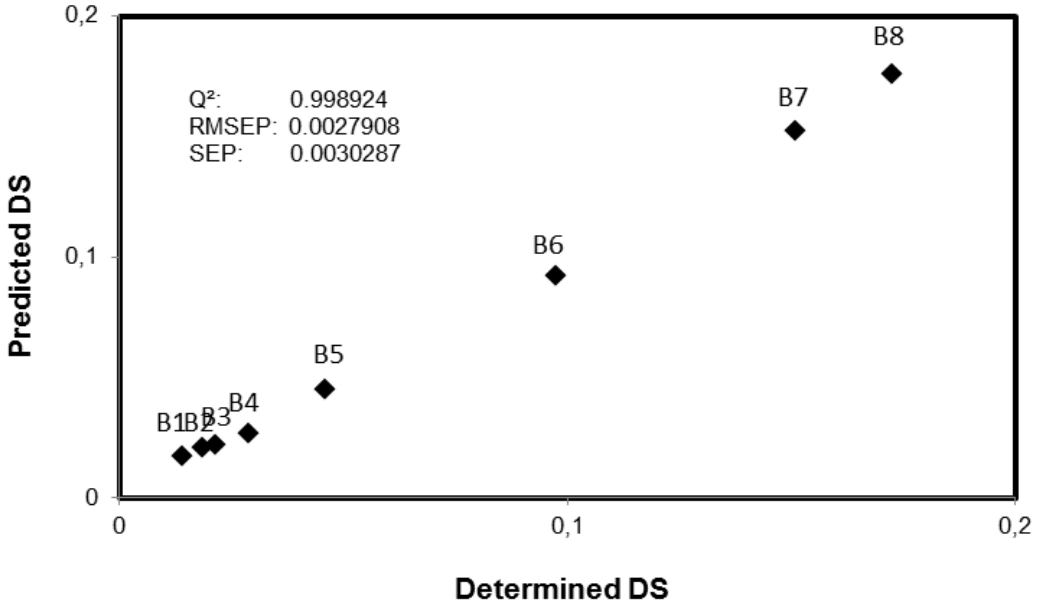

Fig. 4. Predicted *vs*. determined DS values for the birch samples. For abbreviations, see Fig. 3.

Table 3. Cross-validation Results of the PLS Regression Models*

Variable	Q^2	RMSEP	SEP	Rank
P-factor	99.0	9.32	9.53	1
DS	99.8	0.0028	0.0030	1

*Q^2 is the cross-validated coefficient of determination (in cumulative %), RMSEP the root mean standard error of prediction, and SEP the standard error of prediction (in original units) (Davies and Fearn 2006).

TC and TA were the most influential variables according to the loading values of the first latent variables in the PLS model for P-factor, whereas for the second latent variables, TL and TF were the most influential. In the PLS model constructed for DS, the loading values indicated that the most significant variable was TC.

For clarity, and to further illustrate the validity of the used models, the PLS regression models for P-factor and DS were calculated and cross-validated using data corresponding to individual samples. The regression results of these models are shown in Tables 4 and 5.

The calculated *vs.* analytically determined values for P-factor and DS are shown in Figs. 5 and 6. The calculated values are from cross-validated models using individual samples. The Q^2, RMSEP, and SEP values of these models are given in Table 6. It is noted that the values shown were very similar to those shown in Table 3 and Figs. 5 and 6. In addition, the values illustrated in Tables 3 and 6 were slightly closer together in birch samples than they were in the previous study conducted with pine samples (Lehto *et al*. 2014).

Table 4. Predicted *vs.* Determined P-factor Values from Individual Samples*

Sample	Predicted values	Determined values
B1a	12	10
B1b	10	10
B2a	21	20
B2b	18	20
B3a	27	30
B3b	29	30
B4a	35	41
B4b	42	41
B5a	63	60
B5b	57	60
B6a	124	119
B6b	136	119
B7a	157	179
B7b	190	179
B8a	238	238
B8b	236	238

*Calculations have been made using the model incorporating one latent variable. The symbols a and b refer to parallel samples.

Table 5. Predicted *vs*. Determined DS Values (% o.d. Wood) from Individual Samples*

Sample	Predicted values	Determined values
B1a	1.7	1.4
B1b	1.6	1.4
B2a	2.1	1.9
B2b	2.0	1.9
B3a	2.2	2.2
B3b	2.2	2.1
B4a	2.7	2.8
B4b	2.7	3.0
B5a	4.4	4.5
B5b	4.6	4.7
B6a	9.2	9.9
B6b	9.4	9.6
B7a	15.5	15.1
B7b	14.9	15.1
B8a	17.9	17.3
B8b	16.9	17.3
*Calculations have been made using the model incorporating one latent variable. The symbols a and b refer to parallel samples.		

Table 6. Cross-validation Results (Q^2, RMSEP, and SEP Values with Rank of the Model) of the PLS Regression Models Calculated Based on Data from Individual Samples

Variable	Q^2	RMSEP	SEP	Rank
P-factor	98.7	9.52	9.80	1
DS	99.6	0.0038	0.0039	1

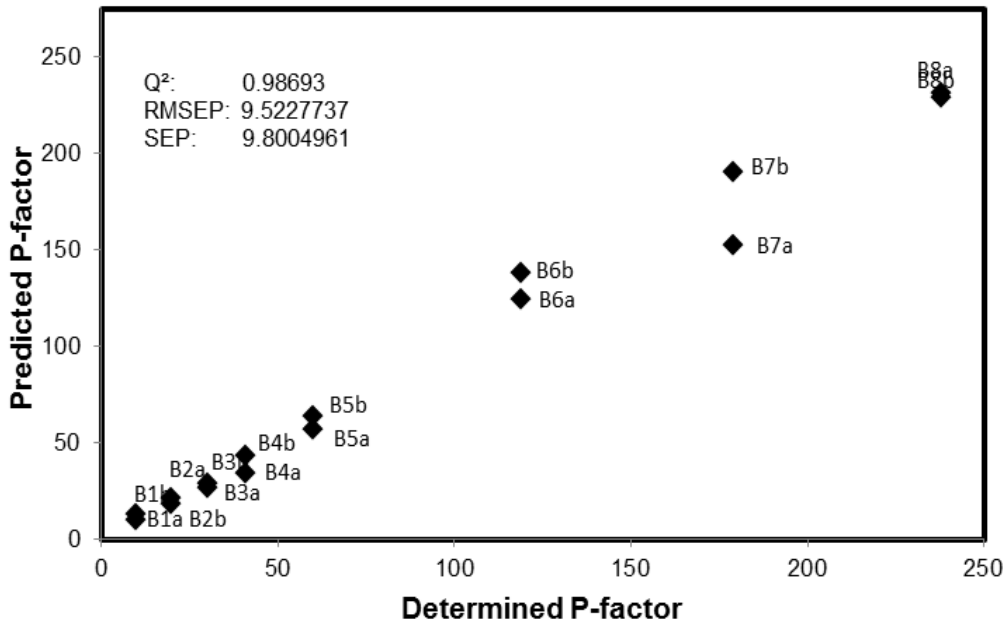

Fig. 5. Predicted *vs*. determined P-factor values based on data from individual samples. Calculations have been made using the model and incorporating one latent variable. For abbreviations, see Fig. 3.

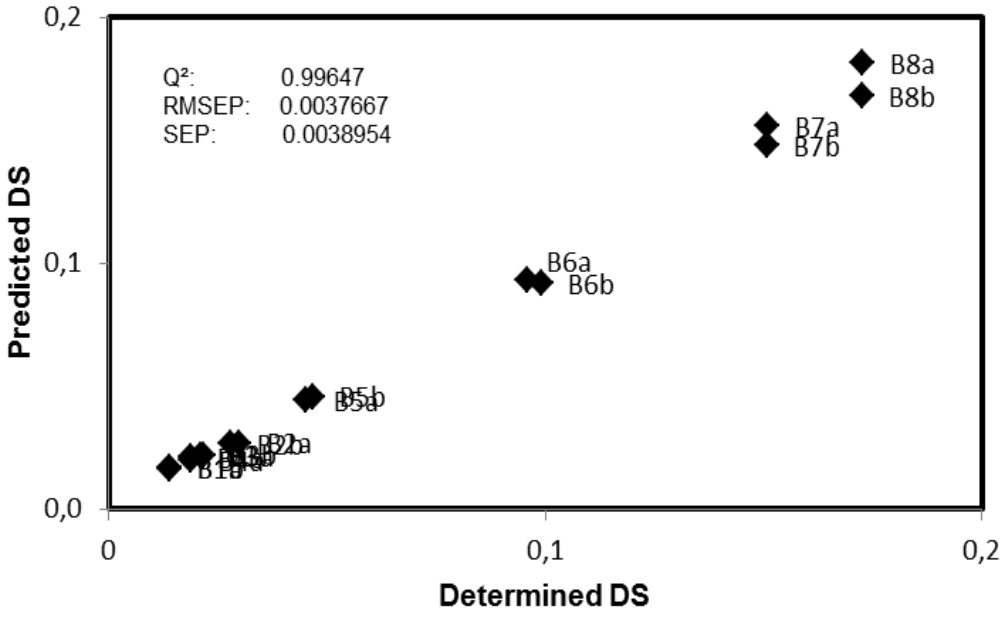

Fig. 6. Predicted *vs*. determined DS values based on data from individual samples. Calculations have been made using the model and incorporating one latent variable. For abbreviations, see Fig. 3.

CONCLUDING REMARKS

1. The autohydrolysis of wood chips offers a potential method for recovering carbohydrate-rich materials prior to pulping, thus creating a possibility for more efficient use of wood and production of value-added chemicals and materials from renewable feedstocks. This approach includes the modification of a modern pulp mills into an integrated forest biorefinery (IFBR). However, the process parameters and modification of the mill environment must be adjusted to meet the requirements set for the overall economics and process efficiency. This target includes a careful and thorough determination of the effects of different sub-processes on the overall efficiency of the total process concept. The chemometric approach shown in this study provides a useful tool for further understanding the effects of variations in time and temperature during the autohydrolysis of birch chips.
2. Nordic pulp mills utilize both hardwoods and softwoods for the production of pulp. Due to the fact that wood species vary in the chemical composition and behavior during applied pulping conditions, each process step has been designed in such a way that the value extracted from available feedstocks is maximized. However, the integration of additional biorefinery processes into an existing pulp mill requires the same evaluation of the additional process parameters. The behavior of wood species with different chemical characteristics can be fractionated into valuable product streams (in this case hydrolysates) containing organic fractions in various ratios. In addition to carbohydrates (monomeric, oligomeric, and polymeric substances) and carbohydrate-derived degradation products (organic acids and furanoic compounds), the hydrolysates contain some lignin- and extractives-derived materials. As the chemical compositions of the produced hydrolysates are

strongly characteristic of used feedstock materials, the chemometric models are also different between the used wood species. For this reason, the chemometric analyses performed with two types of pulp woods showed distinct features unique for each species and separate multivariate models for each material are needed.

3. Chemometric techniques have been carried out for description of various forest industry processes. However, although many pulping applications have been extensively studied, only a limited amount of research has been conducted with the data concerning IFBR concepts, such as autohydrolysis of the common pulp woods. When regarding the overall efficiency and economy of the integrated processes, the need for rapid and reliable method for the proper interpretation and utilization of chemical data is highly appreciated. By such basic chemometric techniques such as PCA and PLS, relatively accurate models could be constructed for autohydrolysis processes conducted with different wood species. In addition to the rapid process description, the chosen method of data analysis gives plenty of visual information about the affected parameters *via* the graphical presentation of model scores and different loadings.

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