Optimization of Acid Pretreatment in order to Increase the Phenolic Content of *Picea abies* Bark by Surface Response Methodology

Xavier Duret,[a,b,d,*] Emmanuel Fredon,[a,b,*] Eric Masson,[d] Lyne Desharnais,[a] and Philippe Gérardin[a,c]

The purpose of this work was to determine the main factors influencing the phenolic content of bark during acid hydrolysis. The optimization of polysaccharides hydrolysis was done by response surface methodology. The hydrolysis was performed under atmospheric pressure in an aqueous solution of sulfuric acid. An experimental design was applied to analyze the effects of the reaction time (5 to 24 hours), acid concentration (3 to 20%), and solid/liquid ratio (1/10 to 1/5) on the weight loss, lignin content, holocellulose content, and sugar yield for the hydrolysis. The pretreated bark had a high lignin content of 60% resulting from hemicelluloses hydrolysis and phenolic compound condensation.

Keywords: Norway spruce; Bark; Acid hydrolysis; Phenolic content optimization; Response surface methodology

Contact information: a: Université de Lorraine, Laboratoire d'Etudes et de Recherche sur le Matériau Bois (LERMAB), Nancy, EA 4370, F-54506 France; b: ENSTIB, 27 rue Philippe Séguin, BP 1041, 88051 EPINAL Cedex 9, France; c: Faculté des Sciences et Technologies, BP 70239, 54506 Vandœuvre les Nancy, France; d: Centre de ressources pour les industries du bois (CRITT Bois), 27 rue Philippe Séguin, B.P 91067 88051, EPINAL cedex 9, France;
** Corresponding authors: rhodanis@wanadoo.fr; emmanuel.fredon@enstib.uhp-nancy.fr*

INTRODUCTION

Phenolic resins for wood products have been derived largely from petroleum, a finite natural resource. Some efforts have been devoted to the development of thermosetting phenol-formaldehyde type adhesives from vegetable origins. The best way is the use of tannins or lignin to replace a part of the phenol (Pizzi and Mittal 2003; Yasaki and Collins 1994a,b; Navarette *et al.* 2012). Bark is a low-value wood by-product of sawmills and paper mills, which is a readily available and renewable resource, amounting to about 7% of the total weight of a tree and 12% of the total volume of a tree (Florentin 2011). Investigation of alternative higher value added uses of bark could be economically advantageous according to the biorefinery concept. Moreover, it brings a new contribution to the attempt concerning the climate change by incorporating the carbon from a photosynthetic material into a durable product. The naturally higher phenolic content of bark and lower polysaccharides content relative to wood is convenient for the phenolic thermosetting resins synthesis. Nevertheless, the still remaining carbohydrate fraction is known to induce a negative effect on the curing of the resins, mechanical properties, and durability (Christiansen and Gillespie 1986; Pilato 2010; Yang *et al.* 2006). The aim of this study was to increase the phenolic content (mainly tannins and lignin) in *Picea abies* bark solids remaining after pretreatment with acid hydrolysis. As a consequence, the best way to eliminate the carbohydrate fraction in

biomass is to degrade them. The polysaccharides can be hydrolyzed by thermal, chemical, or enzymatic reaction (Girio *et al.* 2010; Matsushita *et al.* 2010; Mehrotra *et al.* 2010). Bark is a source of phenolic compounds that are enzymatic inhibitors in the enzymatic hydrolysis, and the thermal treatment induces important chemical changes (Tejirian and Xu 2011). These are the reasons why the pretreatment needs to be performed under acid hydrolysis. Optimization of the phenolic content of bark for chemical feedstock could be a better way to upgrade the spruce bark than ethanol production. The pretreated bark will be liquefied and used to synthesize thermosetting resins, which can be used as adhesives (Pan 2007), foams (Alma and Shiraishi 1998), films (Kurimoto *et al.* 2001), composites (Kishi and Fijita 2008), or wood impregnation to improve its durability.

EXPERIMENTAL

Materials

Anhydrous *Picea abies* bark particles were used. The bark was collected in a sawmill of Vosges Department of France. Samples were dried at 103°C until constant weight. Results are based on the anhydrous weight of bark. For higher scale this step will be not necessary. Nevertheless, low moisture is necessary to prevent the degradation of bark in the stock. Bark was milled to pass through a sieve of 400 µm.

Extractive Content

The extractive content of *Picea abies* bark was determined by successive extractions. The solvents were petroleum ether, a mixture of toluene/ethanol (80/20), and water. The extraction was carried in soxhlet apparatus with more than four cycles per hour for 9 h. The extractive content is calculated as a percentage based on the weight of anhydrous bark.

Total Phenolic Content of Extracts

Analysis of total phenolic content of bark was performed employing the literature method involving Folin-Ciocalteu reagent and gallic acid as standard (Wilfred and Ralph 2006). The reaction is based on the reduction of phosphomolybdic acid by phenols in aqueous alkali, allowing determination of total soluble phenolic compounds. The sample was diluted in water (10 mg/mL). Two milliliters of freshly prepared 2% (w/v) of sodium carbonate was added to 0.1 mL of dilute sample and mixed on a vortex mixer. After 5 min at 25°C, 0.1 mL of 1:1 dilution of Folin-Ciocalteu reagent was added. After 30 min at 25°C, the absorbance was read at 750 nm. Gallic acid was used to generate a calibration curve. The results were then reported as gallic acid equivalent per gram of the extract.

Holocellulose Content

The holocellulose content was determined by the chlorite method (Rowell 2005). To 1 g of the sample, 32 mL of hot distilled water was added with 200 µL of acetic acid and 0.4 g of sodium chlorite. The mixture was heated in a water bath at 75°C. After each hour, a fresh portion of 200 µL of acetic acid and 0.4 g of sodium chlorite was added with shaking. The addition of acetic acid and sodium chlorite was repeated until total delignification and the mixture was let in the water bath at 75°C overnight without

further addition of acetic acid and sodium chlorite. After the reaction, the sample was cooled, filtrated on a sintered glass number 3, and washed with acetone and water. Holocellulose was dried at 105°C until constant weight. The holocellulose content was based on the weight of bark without treatment.

Cellulose and Hemicelluloses Content

The hemicelluloses and α-cellulose content was determined by treatment of holocellulose with sodium hydroxide solution and then with acetic acid according to the procedure described by Rowell (2005). Cellulose content was directly calculated from dry residue weight. One-half grams of anhydrous holocellulose was placed into a glass beaker. Two and a half milliliters of 17.5% NaOH solution was added to the holocellulose. A glass rod was used to manipulate lightly the holocellulose until it became soaked with NaOH solution. After the first addition of NaOH solution, 1.25 mL of 17.5% NaOH solution was added three times at 5 min intervals. The mixture was kept at 20°C for 30 min. Then 8.25 mL of distilled water was added, and the mixture was kept at 20°C for 1 h. The cellulose was filtered with a fritted glass crucible of medium porosity with suction and washed with 25 mL of 8.3% NaOH solution. Then the residues were washed with distilled water; 3.75 mL of 10% acetic acid solution was added into the crucible without suction for 3 min. Then, the suction was applied to filter the residues. The residues were washed for the last time with distilled water until the cellulose was free of acid as indicated by pH paper. The residues were dried at 103°C until constant weight. The cellulose content was expressed as the ratio of residues weight on the initial mass of anhydrous bark (with extractive content). The hemicellulose content was deduced from the cellulose content and holocellulose content of the bark**.**

Klason Lignin Content

Klason lignin is acid-insoluble lignin (ASTM standard D1106 - 96, 2007). A first hydrolysis was achieved in 72% sulfuric acid at 30°C for 60 min. For 0.1 g of the sample, 1.5 mL of 72% sulfuric acid solution was added. Then the reaction mixture was diluted to obtain 3% sulfuric acid and heated at 120°C in an autoclave for 1 h. The reaction mixture was filtered in sintered glass filter number 3 and washed with hot water. The solid residues were dried at 105°C overnight and weighted. The yield of acid insoluble lignin was based on oven dried original bark (%).

Bark Hydrolysis

Bark was hydrolyzed in aqueous solution of sulfuric acid. The unwashed bark was mixed with an acid solution and heated in oil bath at 100°C, under reflux, with a magnetic stirring. The reaction time, acid concentration, and solid/liquid ratio are set by the experimental design (Table 1).

Table 1. Experimental Domain of Central Composite Design

Factors	Levels				
Coded Values	-1.668	-1	0	1	1.668
Reaction Time	1 hour	5.6 hours	12.5 hours	19.4 hours	24 hours
Acid Concentration	3%	6.4%	11.5%	16.6%	20%
Solid/Liquid Ratio (w/w)	0.01	0.0382	0.08	0.1219	0.15

At the end of the reaction time the mixture was cooled in an ice bath to stop the reaction. The mixture was filtrated under reduced pressure on a glass fiber filter. The bark was rinsed with distilled water. The hydrolyzed bark was dried to a constant weight at 105°C. The hydrolysate was completed to 1 L with distilled water and was kept refrigerated for further analysis. The mass loss was expressed as the ratio of the mass of bark before and after hydrolysis.

HPAEC-PAD Analyses

Separation and quantification of neutral sugars were performed using a Dionex ICS-3000 system consisting of a SP gradient pump, an AS auto sampler, an ED electrochemical detector with a gold working electrode, an Ag/AgCl reference electrode and Chromeleon version 6.8 (Dionex Corp., USA). A Carbopac PA1 (4 x 250 mm, Dionex) column with a guard column (4 x 50 mm, Dionex) was used as a stationary phase using isocratic conditions with 1 mM sodium hydroxide as the eluent. Eluents were prepared by dilution of a 46 to 48% NaOH solution (PA S/4930/05 Fisher Scientific) in ultra-pure water. All eluents were degassed before use by flushing helium through for 20 min; subsequently they were kept under a constant helium pressure (eluent degassing module, Dionex). After each run, the column was washed for 10 min with 200 mM NaOH solution and reequilibrated for 15 min with 1 mM sodium hydroxide. Samples were injected through a 25 µL full loop and separations were performed at 25°C at a rate of 1 mL/min. The pulse sequence for pulsed amperometric detection consisted of a potential of +100 mV (0-200 ms), +100 mV integration (200-400 ms), -2000 mV (410-420 ms), +600 mV (430 ms), and -100 mV (440-500 ms). The results were expressed as a percentage of glucose and non-glucosidic sugars based on the initial mass of anhydrous bark.

Response Surface Methodology

In order to determine the optimal conditions to obtain a maximum phenolic content in the bark, an experimental design with response surface methodology was applied. This experimental design allows studying a phenomenon that can be explained by a quadratic model (Equation 1). β_0 is a constant coefficient, β_i is the coefficient of the factors i, X_i is the level of the factor i, X_j is the level of the factor j, $\beta_{i,j}$ is the coefficient of the first order interaction between the factor i and j, and $\beta_{i,i}$ is the coefficient of the quadratic term of the coefficient i. Three factors determined from a previous experimental design were studied, and their effects on the weight loss, holocellulose content, and Klason lignin yield were determined. For this experimental design, the experimental domain is presented in the Table 1. The experimental design was a central

composite design, and it is showed in Table 4. The results were analyzed using the statistic software R (R Development Core Team 2011). The optimal region were calculated through the first derivate of the mathematical function. Thus, the optimal point was determined by solving the first grade system formed by first derivate equations equal to zero.

$$y = \beta_0 + \sum_{i=0}^{k} \beta_i x_i + \sum_{i=0}^{k} \beta_{ij} x_i x_j + \sum_{i=0}^{k} \beta_{ii} x_i^2 \quad (1)$$

RESULTS AND DISCUSSION

Characterization of Chemical Bark Composition

The chemical composition of Norway spruce bark is shown in Table 2. Values were examined in the objective of enrichment of phenolic part and minimization of polysaccharides. For this purpose, fresh bark was separated following inner and outer bark. Each of them was analyzed for its main components, cellulose, hemicelluloses, lignin, and total phenol content of extracts. The holocellulose content of inner bark and outer bark were quite similar, with 51.4 and 48.9%, respectively. But outer bark also contained more lignin (32%) than the inner bark (12%). Nevertheless, the Klason lignin of bark is composed of lignin and condensed tannins which are not extracted by the common extraction process.

Table 2. Chemical Composition of *Picea abies* Bark

	Extractives (%)*[1]		Cellulose (%)*		Hemicellulose (%)*		Klason Lignin (%)*	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
Whole Bark	23.3	0.5	29.0	0.91	21.7	0.66	24.5	0.21
Inner Bark	32.1	0.75	25.4	0.79	26.0	1.05	12.0	0.05
Outer Bark	15.6	0.44	32.8	1.02	16.1	0.97	32.8	0.84
*:Based on the weight of anhydrous bark with extractives								
[1] :Extractives from petroleum ether, toluene/ethanol and water extract								

Moreover, bark extracts are known to be constituted of non-cellulosic polysaccharides, stilbene glucosides, low molecular weight phenolic compounds, and condensed tannins (Krogell *et al.* 2011). The inner bark extractives content was twice that of outer bark. Nevertheless, inner bark had lower total phenolic content in water extracts than outer bark with 276 mg/g and 436 mg/g, respectively (Table 3). Phenolic compounds are

a predominant fraction in bark extracts: The yield of total phenols in the toluene/ethanol and water bark extracts were 773 mg/g (gallic acid equivalent) and 401 mg/g, respectively (Table 3). In toluene ethanol extracts, inner bark contained as much phenolic compounds as outer bark. In water extracts, inner bark contains less than outer bark. Krogell *et al*. (2011) indeed found, in inner bark water extracts, predominantly phenolic extractives such as stilbene glucosides and non-phenolic extractives such as saccharides. Most of the condensed tannins, which have a great importance in resin formulation, are found in the inner bark.

Table 3. Total Phenolic Content of *Picea abies* Bark extracts

		Average*	Standard Deviation
Toluene/Ethanol	Bark	772.6	0.2
	Outer Bark	775.5	0.3
	Inner Bark	758.8	0.2
Water	Bark	401.6	0.03
	Outer Bark	436.1	0.05
	Inner Bark	276.3	0.04

*: gallic acid equivalent (mg/g of extracts)

Finally, the difference in global phenolic and carbohydrate parts between outer and inner bark was not important enough to discard one of the fractions. Then, the whole pretreated bark will be used for the thermosetting resins formulation. Nevertheless, it is advantageous to remove the carbohydrate fraction in order to enhance the thermosetting ability.

Response Surface Experimental Design Analysis

Response surface methodology was used to optimize the phenolic content of spruce bark. This method allows finding the quadratic model describing the responses with the factors that were investigated. This kind of experimental design allows for the study of second order phenomena. The response surfaces are presented with only the significant effects. The results of central composite experimental design are presented in Table 4. The analysis of variance and coefficients of response surface are presented in Table 5. The R^2 adjusted was acceptable for studied surfaces responses. Nevertheless, caution should be taken with the surface responses of holocellulose due to a low R^2 adjusted.

Table 4. Factors, Coded Levels, and Responses of Central Composite Experimental Design

Run	Experimental Factor			Responses					
	Reaction Time	Acid Concentration	Solid/Liquid Ratio	Mass Loss*	Klason Lignin*	Total Sugars*	Glucose*	Non-glucosidic Sugars*	Holocellulose*
1	-1	-1	-1	0.3415	0.5013	0.1725	0.0716	0.1010	0.3817
2	-1	-1	1	0.3500	0.5080	0.1839	0.0669	0.1170	0.3413
3	-1	1	-1	0.4014	0.5666	0.1956	0.0794	0.1161	0.2995
4	-1	1	1	0.3627	0.5438	0.1932	0.0790	0.1142	0.3284
5	1	-1	-1	0.3885	0.5139	0.2023	0.0790	0.1233	0.3455
6	1	-1	1	0.3073	0.5163	0.1959	0.0746	0.1213	0.3601
7	1	1	-1	0.4151	0.5865	0.1584	0.0811	0.0773	0.3021
8	1	1	1	0.3180	0.5877	0.1607	0.0746	0.0860	0.2980
9	-1.668	0	0	0.3033	0.5114	0.1782	0.0643	0.1139	0.3870
10	1.668	0	0	0.3754	0.5921	0.1690	0.0783	0.0907	0.3199
11	0	-1.668	0	0.3418	0.5007	0.1898	0.0693	0.1205	0.3870
12	0	1.668	0	0.3732	0.6067	0.1479	0.0802	0.067	0.2815
13	0	0	-1.668	0.4279	0.5463	0.1825	0.0775	0.1050	0.3615
14	0	0	1.668	0.3496	0.5791	0.1827	0.0766	0.1061	0.2939
15	0	0	0	0.3856	0.5895	0.1818	0.0743	0.1074	0.3235
16	0	0	0	0.3895	0.5768	0.1853	0.0769	0.1084	0.3035
17	0	0	0	0.3850	0.5791	0.1891	0.0775	0.1115	0.3389
18	0	0	0	0.3790	0.5761	0.1868	0.0770	0.1098	0.3112
19	0	0	0	0.3822	0.5724	0.1813	0.0766	0.1047	0.3241
20	0	0	0	0.3783	0.5831	0.1870	0.0771	0.1099	0.3375
21	0	0	0	0.3807	0.6010	0.1924	0.0790	0.1134	0.3391
22	0	0	0	0.3801	0.5706	0.1922	0.0783	0.1139	0.3294
23	0	0	0	0.3796	0.5998	0.1866	0.0769	0.1096	0.3040

*: Based on the weight of anhydrous hydrolyzed spruce bark with extractives

The F test value for variance analysis was good with a p-value lower than 0.01. The F test value for the lack of fit was good with a p-value lower than 0.02, except for Klason lignin content and holocellulose content. A p-value lower than 0.05 is usually taken as a limit, but a p-value of 0.1 is still significant. Holocellulose content and Klason lignin content could be fitted better with another model (third order), (Table 4).

Table 5. Analysis of Central Composite Experimental Design for Quadratic Model

Coefficients	Mass Loss	Klason Lignin	Total Sugars	Glucose	Non-Glucosidic Sugars	Holocellulose Content
β_0	0.3822	0.5835	0.1867	0.0770	0.1097	0.3237
β_1	0.0069 °	**0.0162** **	-0.0032	**0.0026** ***	**-0.0058** **	**-0.0116** *
β_2	**0.0120** **	**0.0311** ***	**-0.0086** ***	**0.0030** ***	**-0.0116** ***	**-0.0278** ***
β_3	**-0.0250** ***	0.0031	0.0004	**-0.0013** *	0.0017	-0.0084
β_{12}	-0.0044	0.0054	**-0.0139** ***	**-0.0022** *	**-0.0117** ***	-0.0013
β_{13}	**-0.0185** **	0.0025	-0.0016	-0.0007	-0.0009	0.0028
β_{23}	-0.0079	-0.0038	-0.0006	0.0003	-0.0009	0.0633
β_{11}	**-0.0154** ***	**-0.0141** **	-0.0029	**-0.0017** **	-0.0012	0.0092 °
β_{22}	**-0.0088** *	**-0.0134** **	**-0.0047** *	-0.0005	**-0.0042** *	0.0023
β_{33}	0.0024	**-0.0102** *	0.0003	0.0003	-0.00003	-0.00008
R^2	0,8914	0,9009	0,8471	0.8461	0.8762	0.752
R^2 Adjusted	0,8163	0,8322	0,7413	0.7395	0.7904	0.5803
Residual Standart Error	0.0135	0.0145	0.0067	0.0022	0.0064	0.0195
Variance analysis						
F_{exp}	11,86	13.13	8.00	7.94	10.22	4.38
Prob>F	0.0001	<0.0001	0.0005	0.0005	0.0001	0.0083
Lack of fit						
F_{exp}	32.25	2.68	5.88	5.62	11.05	3.22
Prob>F	<0.0001	0.1037	0.0143	0.0162	0.0020	0.0689
Significant Factors: P value<0.001=***; P value<0.01=**; P value<0.05=*; P value<0.1=°.						

Mass loss analysis

Figure 1 shows the response surface of the mass loss. The mass loss is represented as a function of reaction time and acid concentration. One response surface is presented for each solid/liquid ratio levels of -1.668, 0, and 1.668. The most significant effects were the solid/liquid ratio (β3) and the quadratic effect of the reaction time (β11). The acid concentration (β2) and the interaction between reaction time and solid/liquid ratio were also significant (β13). The reaction time (β1) and the quadratic effect of the acid concentration (β22) had low effect on the mass loss. The interactions β12, β23, and the quadratic effect of solid/liquid ratio (β33) were negligible (Table 5). The mass loss as a function of reaction time reached a maximum. The optimal reaction time did not change greatly with the acid concentration, but the mass loss was higher when the acid concentration increased. The optimal reaction time and the mass loss were greatly influenced by the solid/liquid ratio. The optimal reaction time was longer and the mass loss was higher when the solid/liquid ratio was lower. Then, the interaction between reaction time and solid/liquid ratio was significant and was the only significant interaction. The non-negligible quadratic effect of the reaction time on the mass loss could be explained by condensation reactions resulting from degradation products of

carbohydrate as furfural (Nguyen *et al.* 1999; Sakostschikoff *et al.* 1934; Stanciu and Ciurea 2008) and condensed tannins polymerization under acidic conditions (Evelyn *et al.* 1960).

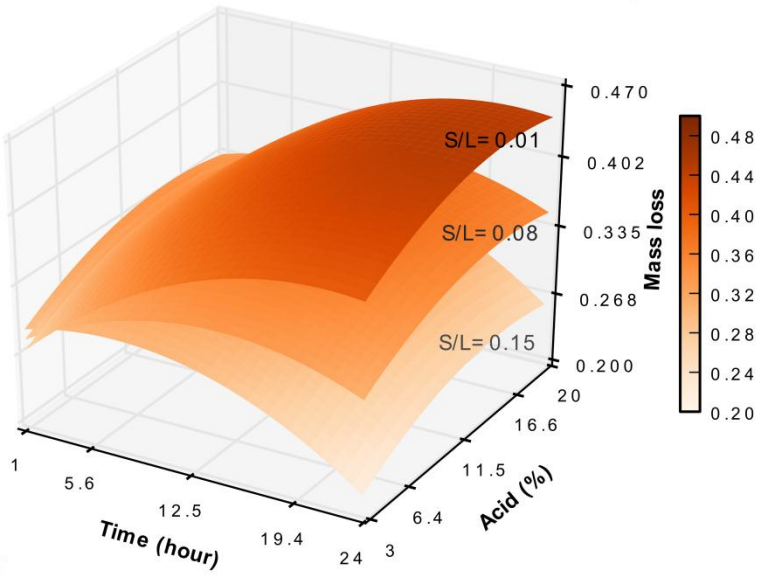

Fig. 1. Response surface of mass loss

Klason lignin content

The Klason lignin of hydrolyzed barks is composed of lignin and condensed tannin. Figure 2 shows the response surface of the Klason lignin content, represented as a function of reaction time and acid concentration.

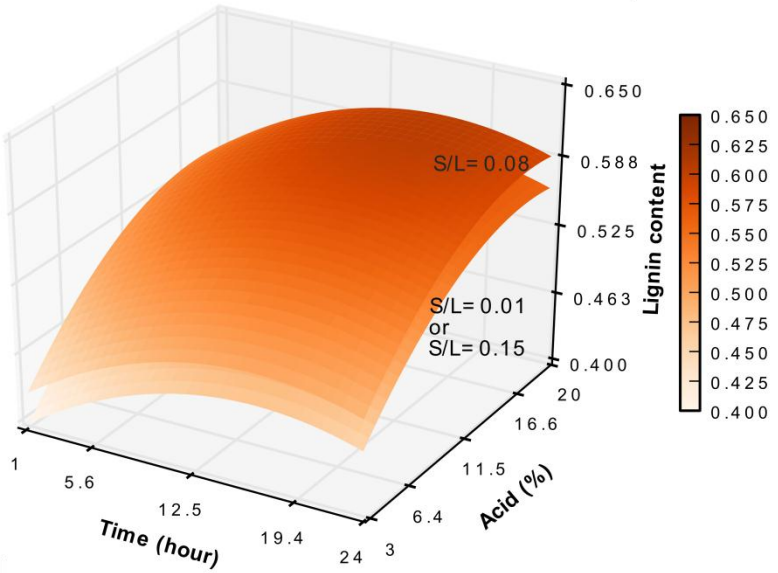

Fig. 2. Response surface of lignin content

Response surfaces are presented for the following solid/liquid ratio levels: -1.668, 0, and 1.668. The levels -1.668 and 1.668 are represented by the same surface. The Klason lignin content was most significantly influenced by the main effect of acid

concentration (β1), then by the main effect of reaction time (β2) and all the quadratic effects (β11, β22, β33). The interactions and the solid/liquid ratio were negligible (Table 5). Klason lignin content increased with high acid concentration and reached a maximum of 60% at optimal reaction time.

Holocellulose content

Figure 3 shows the response surface of the holocellulose content. The holocellulose content is represented as a function of reaction time and acid concentration. The holocellulose content was mainly influenced by the main effect of reaction time (β1) and acid concentration (β2), and also the quadratic effect of the reaction time (β11). The effects of solid/liquid ratio (β3), the interactions (β12, β13, β23), the quadratic effects of the acid concentration (β22), and the solid/liquid ratio (β33) were negligible. The acid concentration (β2) is the most significant factor (Table 5). Holocellulose content clearly decreased with increasing of reaction time and acid concentration due to hydrolysis of polysaccharides.

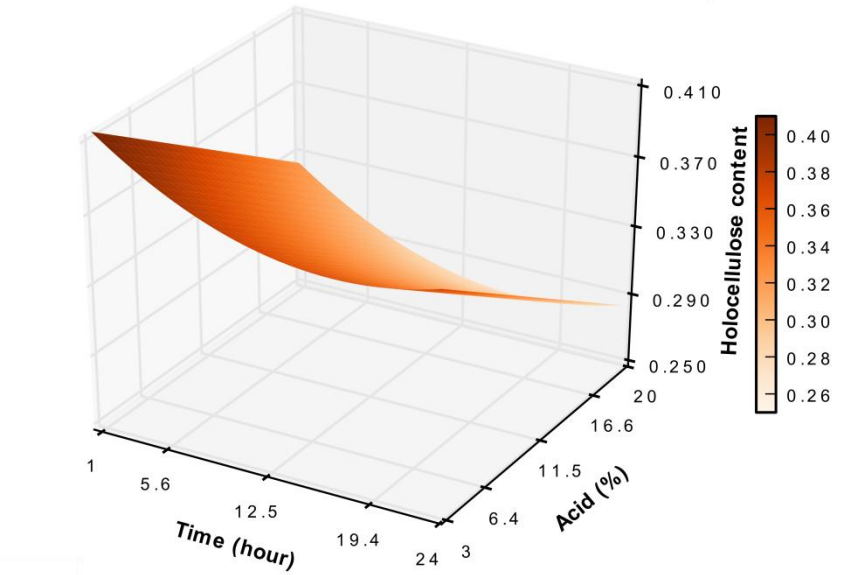

Fig. 3. Response surface of holocellulose content

Total sugar recovery

Figure 4 shows the response surface of sugar recovery. The sugar recovery in hydrolysate is represented as a function of reaction time and acid concentration. The sugar recovery was mainly influenced by the acid concentration, the interaction between reaction time and acid concentration (β12), and the quadratic effect of acid concentration (β22). The other coefficients were negligible (Table 5). For a low acid concentration, the sugar recovery increased with increasing of the reaction time. For a high acid concentration, the sugar recovery decreases with increasing of the reaction time. That is the reason why the interaction between reaction time and acid concentration is so important. The decreasing of sugar recovery is due to furfural formation from sugar degradation reaction.

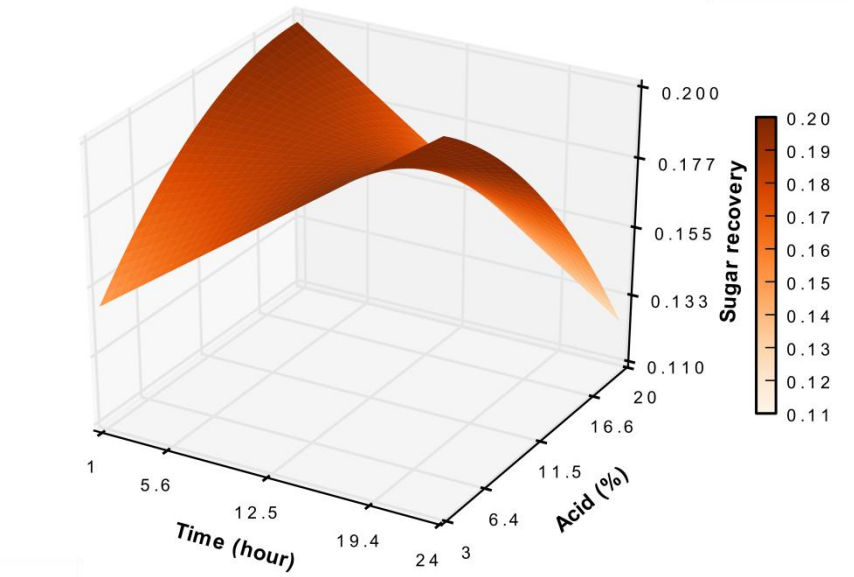

Fig. 4. Response surface of sugar recovery

Glucose and non-glucosidic sugars recovery

Figures 5 and 6 show the response surface of glucose and non-glucosidic sugars from hemicelluloses, respectively. The glucose and other sugars are represented as a function of reaction time and acid concentration. The glucose content of hydrolysate after bark hydrolysis was influenced by the reaction time, the acid concentration, the solid/liquid ratio, the interaction between the reaction time and the acid concentration, and also the quadratic effect of the reaction time. The other effects were negligible. Variations of the glucose content were low. Cellulose is difficult to hydrolyze. The effect of the acid concentration was higher for a short reaction time. The effect of reaction time was also higher for a low acid concentration. This means that the interaction between reaction time and acid concentration was important.

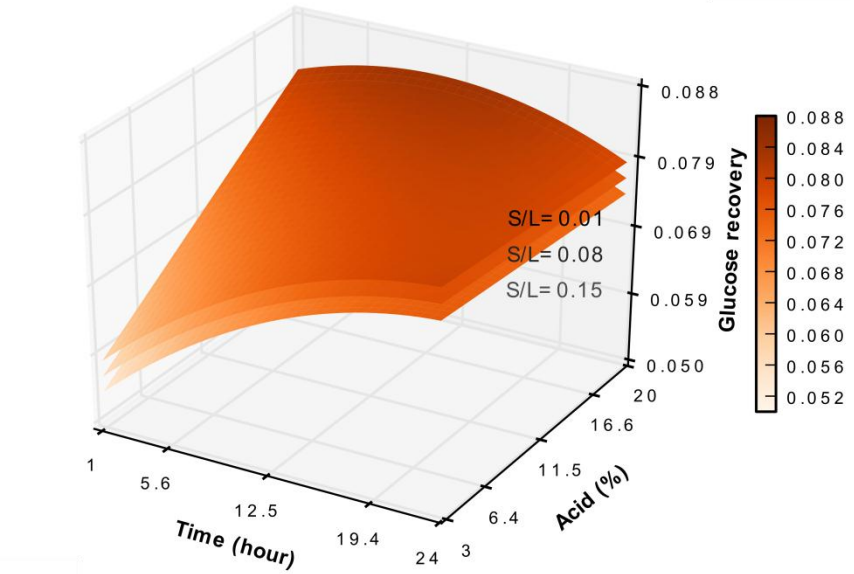

Fig. 5. Response surface of glucose recovery

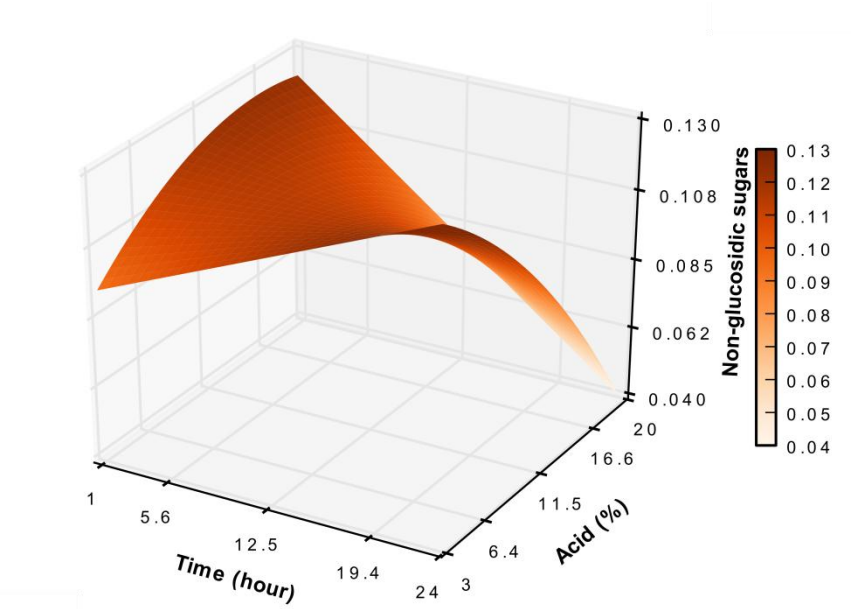

Fig. 6. Response surface of non-glucosidic sugars recovery

The sugars from hemicellulose content of hydrolysate after bark hydrolysis is influenced by the reaction time, acid concentration, the interaction between the reaction time and acid concentration, and the quadratic effects of acid concentration. Other effects like solid/liquid ratio are negligible. The response surfaces of total sugar recovery are mainly influenced by the hemicelluloses hydrolysis. The variation of the glucose content in hydrolysate is low. High reaction time and high acid concentration enhance the sugar degradation.

Extractive content

The extractives content of the initial bark was 23.3% and decreased to about 15% after hydrolysis. The extractives content of hydrolyzed spruce bark could not be described by the model because the adjusted R^2 value was too low. The extractives content did not seem to be influenced significantly by the factors in the experimental domain that was investigated.

Response Surface Experimental Design Analysis

The optimized conditions are based mainly on the Klason lignin and holocellulose content of bark due to the condensation of phenolic compounds such as tannins under acidic condition and non-extractive condensed tannin (Evelyn *et al.* 1960; Krogell *et al.* 2011). The optimized conditions were a reaction time of 18.12 h, an acid concentration of 18%, and a solid/liquid ratio of 0.08. A total hemicelluloses hydrolysis is reached under reflux at 100°C with these optimal conditions. The final chemical composition of spruce bark reached 60% of lignin content. The holocellulose content was quite similar to the cellulose content of initial bark, and the extractives content reached 15%.

The optimized holocellulose content was 28%. It was lowered to the cellulose content of initial spruce bark (29%). It can be deduced that cellulose is not totally hydrolyzed in our experimental domain, due to its high crystallinity. Amorphous cellulose may contribute to a small part of the holocellulose hydrolysis. Cellulose hydrolysis under acidic conditions would require a two-step hydrolysis with concentrated acid and dilute acid, or a hydrolysis reaction in a pressurized reactor at high temperature

with dilute acid (Taberzadeh and Karimi 2007). On the contrary, all of the hemicelluloses seemed to be degraded. Otherwise, glucose recovery showed a low variation, as indicated in Fig. 5. It may be released by hemicelluloses such as glucomannans and storage polysaccharides such as starch. The non-glucosidic sugars of *Picea abies* bark are composed of arabinose, mannose, galactose, and xylose. The predominant sugar in the *Picea abies* bark was arabinose with approximately 4%. The yield of galactose, mannose, and xylose were close to 2%.

Comparison of surfaces response between total sugars, glucose, and other sugar content shows that sugars recovery was mainly influenced by the hemicellulose hydrolysis. In our experimental domain, both reaction time and acid concentration led to strong decreases in sugar recovery, which has been correlated to furfural formation. This compound is known to be able to condense with some phenolic compounds such as tannins and lignin (Nguyen *et al.* 1999; Sakostschikoff *et al.* 1934; Stanciu and Ciurea 2008).

The total phenolic content release during acid hydrolysis is low. Reported concentration of aromatic/phenolic compounds is normally a few milligrams per liter. This is due to the low solubility of phenolic compounds and low degradation of lignin during the hydrolysis process (Taberzadeh and Karimi 2007). The determination of total phenolic compounds for a low concentration was not reliable due to the effect of the variation in phenolic compounds composition which can influence the efficiency of the reduction of the Folin-Ciocalteu reagent (Wilfred and Ralph 2006).

Extractives were decreased to 15%: some extractives, especially hydrophilic compounds were released during the hydrolysis. Tannins are subject to self-condensation due to the acidity of the hydrolysis solution (Evelyn *et al.* 1960; Krogell *et al.* 2011). Condensed tannins under heat and mineral acid produce compounds like phlobaphenes that are insoluble in water (Hemingway and Dward Laks 1992).

The results show an important effect of the solid/liquid ratio on the mass loss. Nevertheless, this factor had low effects on the other responses. This could be explained by the addition of the low effects on the holocellulose content, Klason lignin, and sugar recovery. Tannins condensation and condensation between phenolic compounds and furfural can explain that mass loss is not optimal when most severe conditions are applied. According to the low effect of the solid/liquid ratio on the Klason lignin and holocellulose content, it will be good to keep high solid/liquid ratio in order to reduce the water and energy consumption.

Our optimal conditions are not usual in the hydrolysis process because of the low resulting sugar recovery. For example, the optimal conditions of hemicellulose hydrolysis of sorghum straw in hydrochloric acid were 6% HCl at 100°C, 83 min for a hydrolysis of 88% of theoretical xylose (Herrera *et al.* 2004). Under this condition, 88% of hemicelluloses could be hydrolyzed in our experimental design. In our experimental domain, the simple sugar recovery was optimized under low acid concentration and long reaction time (3% sulfuric acid for 24 h) and also under high acid concentration with a short reaction time (20% sulfuric acid for 1 h). For comparison, our optimal conditions for phenolic content optimization were a high acid concentration for a long time (18% of sulfuric acid for 18 h).

CONCLUSIONS

1. The objective of this study was to optimize the phenolic content of spruce bark. For this purpose, the carbohydrate content was reduced by the acid hydrolysis process. The phenolic content of bark is represented by the Klason lignin content due to the condensation of soluble phenolic compound in the acidic solution, and the non-extractive condensed tannins. The goal was achieved, such that the pretreated bark reached a Klason lignin content of 60% under optimal conditions.
2. Hemicelluloses were totally hydrolyzed and cellulose was not totally degraded, due to its crystallinity.
3. The phenolic compounds such as tannin condensed under the acidic conditions employed, increasing the Klason lignin yield.
4. Compared to usual studies dealing with biomass hydrolysis for ethanol, our work explored unusual experimental domain with more severe conditions leading to degradation of sugars.
5. The pretreated bark is expected to be more convenient for the synthesis of liquefied products based thermosetting phenolic resins because of higher phenolic content.

ACKNOWLEDGMENTS

We acknowledge CG88 (Conseil général des Vosges), ANRT (Agence Nationale de la Recherche et de la Technologie) and the CPeR 2007-2013, Structuration du Pôle de Compétitivité Fibres Grand est (Competitiveness Fibre Cluster) for their financial support.

REFERENCES CITED

Alma, M. H., and Shiraishi, N. (1998). "Preparation of polyurethane-like foams from NaOH-catalyzed liquefied wood," *Holz als Roh-und Werkstoff* 56, 245-246.

ASTM standard D1106 - 96 (2007). "Test method for acid-insoluble lignin in wood," *ASTM International*.

Christiansen, A. W., and Gillespie, R. H. (1986). "Potential of carbohydrates for exterior types adhesives," *Forest Products Journal* 36, 20-28.

Evelyn, S. R., Maihs, E. A., and Roux, D. G. (1960). "The oxidative condensation of (+)-catechin," *Biochemical Journal* 76, 23-27.

Florentin, G. H. (2011). "Mémento 2010-2011," *Technical Report*, Technologic Institute FCBA, France.

Girio, F. M., Fonseca, C., Carvalheiro, F., Duarte, L. C., Marques, S., and Bogel Lukasik, R. (2010). "Hemicellulose for a fuel ethanol: A review," *Bioresource Technology* 101, 4775-4800.

Hemingway, R. W., and Dward Laks, P. (1992). *Plants Polyphenols, Synthesis, Properties, Significance*, Springer Science, United States.

Herrera, A., Téllez-Luis, S. J., Gonzalez-Cabriales, J. J., Ramirez, J. A., and Vasquez, M. (2004). "Effect of the hydrochloric acid concentration on the hydrolysis of sorghum straw at atmospheric pressure," *Journal of Food Engineering* 63, 103-109.

Kishi, H., and Fijita, A. (2008). "Wood-base epoxy resins and the ramie fiber reinforced composites," *Environmental Engineering and Management Journal* 7, 517-523.

Krogell, J., Holmbom, B., Pranovich, A., Hemming, J., and Willfor, S. (2011). "Extraction and chemical characterization of Norway spruce inner and outer bark," *Nordic Pulp and Paper Research Journal*, In press.

Kurimoto, Y., Koizumi, A., Doi, S., Tamura, Y., and Ono, H. (2001). "Wood species effects on the characteristics of liquefied wood and the properties of polyurethane films prepared from the liquefied wood," *Biomass and Bioenergy* 21, 381-390.

Matsushita, Y., Yamauchi, K., Takabe, K., Awan, T., Yoshinaga, A., Kato, M., Kobayashi, T., Asada, T., Furujyo, A., and Fukushima, K. (2010). "Enzymatic saccharification of eucalyptus bark using hydrothermal pretreatment with carbon dioxide," *Bioresource Technology* 101, 4936-4939.

Mehrotra, R., Singh, P., and Kandpal, H. (2010). "Near infrared spectroscopic investigation of the thermal degradation of wood," *Thermochimica Acta* 507-508, 60-65.

Navarette, P., Pizzi, A., Pasch, H., and Delmotte, L. (2012). "Study on lignin–glyoxal reaction by MALDI-TOF and CP-MAS 13C-NMR," *Journal of Adhesion Science and Technology* 26(8-9), 1069-1082.

Nguyen, Q. A., Tucker, M. P., Keller, F. A., Beaty, D. A., Connors, K. M., and Eddy, F. P. (1999). "Dilute acid hydrolysis of softwoods," *Apply Biochemistry and Biotechnology* 77-79.

Pan, H. (2007). "Wood liquefaction in the presence of phenol with a weak acid catalyst and its potential for novolac type wood adhesives," Ph.D. thesis, Louisiana State University and Agricultural and Mechanical College.

Pilato, L. (2010). *Phenolic Resins: A Century of Progress*, Springer, United States.

Pizzi, A., and Mittal, K. (2003). *Handbook of Adhesive Technology*, Marcel Dekker, New York.

R Development Core Team (2011). *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria.

Rowell, R. M. (2005). *Handbook of Wood Chemistry and Wood Composites*, CRC Press, United States.

Sakostschikoff, A. P., Iwanova, W. T., Kurennowa, A. M. (1934). "Determination of pentosans in vegetable containing tannins," *Industrial and Engineering Chemistry* 6, 205-208.

Stanciu, C., and Ciurea, A. (2008). "Research concerning formation, characterization and recovery of lignin polymeric deposits in order to get some lignin-phenol-formaldehyde resins," *Materiale Plastice* 45.

Taberzadeh, M. J., and Karimi, K. (2007). "Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review," *BioResources* 2, 472-499.

Tejirian, A., and Xu, F. (2011). "Inhibition of enzymatic cellulolysis by phenolic compounds," *Enzyme and Microbial Technology* 48, 239-247.

Wilfred, V., and Ralph, N. (2006). *Phenolic Compounds Biochemistry*, Springer Sciences, Netherlands.

Yang, I., Kuo, M., and Myers, D. J. (2006). "Bond quality of soy-based phenolic adhesives in southern pine plywood," *Journal of the American Oil Chemists Society* 83, 231-237.

Yasaki, Y., and Collins, P. J. (1994a). "Wood adhesives from *Pinus radiata* bark," *Holz als Roh-und Werkstoff* 52, 185-190.

Yasaki, Y., and Collins, P. J. (1994b). "Wood adhesives on tannin extracts from barks of some pine and spruce species," *Holz als Roh-und Werkstoff* 52, 307-310.

Article submitted: October 14, 2012; Peer review completed: November 27, 2012; Revised version received and accepted: January 11, 2013; Published: January 23, 2013.