SPRUCE BARK HYDROLYSIS TO OPTIMIZE PHENOLIC CONTENT

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In this study, the optimization of polysaccharides hydrolysis in order to increase the phenolic content of *Picea Abies* bark was investigated by screening experimental design. The high phenolic content of bark was interesting in the thermosetting resins synthesis, but the carbohydrate fraction had a negative effect on the mechanical properties and durability of phenolic resins. The purpose of this paper was to determine the main factors influencing the phenolic content of bark during acid hydrolysis. The hydrolysis was performed under atmospheric pressure in an aqueous solution of sulfuric acid. The effects of the reaction time (5 to 24 hours), acid concentration (3 to 10%), solid/liquid ratio (1/10 to 1/5), and particle sizes on the weight loss, lignin content, holocellulose content, and sugar, as well as degradation products of the hydrolysis, were studied.

Keywords: Norway spruce bark, acid hydrolysis, high phenolic content, experimental design

INTRODUCTION

Phenolic adhesives for wood products have been derived largely from petroleum, a finite natural resource. Some efforts have been devoted to develop thermosetting phenol-formaldehyde type adhesives of vegetable origins. The use of tannins or lignin to replace a part of phenol in the thermosetting resins is often studied.[1,2,3] Bark is a low-value wood by-product. It is a readily available and renewable resource, amounting to about 7-13% of the total volume of a tree. The naturally higher phenolic content and lower poly-saccharides content of bark, compared to wood, are convenient in the phenolic thermosetting resin synthesis. Nevertheless, the carbohydrate fraction has a negative effect on the curing, mechanical properties and durability of resins.[4,5,6] A method to eliminate the carbohydrate fraction in biomass is to degrade it. Polysaccharides can be degraded by thermal, chemical or enzymatic reaction.[7,8,9] Bark is a source of phenolic compounds that are enzymatic inhibitors in the production of ethanol.[11] An optimization of the tannins

and lignin content of bark for chemical feedstock could be better for upgrading spruce bark than ethanol production. The phenolics-enriched lignocellulosic biomass could be liquefied to synthesize resin. The aim of this study has been to optimize the phenolic content of *Picea Abies* bark by hydrolysis of polysaccharides. The pretreated bark will be used to synthesize thermosetting resins, which can be used as adhesives,[12] foams,[13] films,[14] composites[15] or wood preservative.

EXPERIMENTAL

Bark material

Picea Abies bark particles were used in this study. *Picea Abies* bark was collected in a sawmill of the Vosges department of France. The samples were dried, milled and screened. Particle sizes were set by the experimental design.

Extractive content

The yield of extractives of *Picea Abies* bark was determined by successive extraction. The solvents used were petroleum ether, a mixture of toluene/ethanol (80/20), and water. The extraction was carried out by a Soxhlet apparatus, with more than four siphonings per hour for 9 hours. All the chemicals used were reagent-grade and obtained from commercial sources.

Holocellulose content

The holocellulose content was determined by the chlorite method.[16] To 1 g of sample, 32 ml of hot distilled water, 0.2 µL of acetic acid and 0.4 g of chlorite sodium were added. The mixture was heated in water bath at 75 °C. After each hour, a fresh portion of 0.2 µL of acetic acid and 0.4 g of sodium chlorite were added while shaking. The addition of acetic acid and sodium chlorite was repeated until total delignification. After, the mixture was left in a water bath at 75 °C overnight without further addition of acetic acid and sodium chlorite. The sample was then cooled, filtrated on a sintered glass filter (No. 3), and washed with acetone. Holocellulose was dried at 103 °C until constant weight. The holocellulose content was based on the weight of bark with extractive.

Klason lignin content

Klason lignin is acid-insoluble lignin.[17] A first hydrolysis was achieved in 72% sulfuric acid at 30 °C for 60 minutes. Then, the reaction mixture was diluted to obtain 3% sulfuric acid, and heated at 120 °C in autoclave for one hour. The reaction mixture was filtered in a sintered glass filter (No. 3), and washed with hot water. The crucible was dried at 103 °C overnight, and weighed. The yield of acid insoluble lignin was based on dried bark with extractives.

Total phenolic content

The reaction is based on the reduction of phosphomolybdic acid by phenols in aqueous alkali. The method determines the total soluble phenolics. Tannins and many other phenolics are not differentiated. The sample was diluted in water. 2 mL of freshly prepared 2% (w/v) sodium carbonate was added to 0.1 mL of dilute sample and mixed on a vortex mixer. After 5 minutes, 0.1 mL of 1:1 dilution of Folin Ciocalteu reagent was added. After 30 minutes, the absorbance was read at 750 nm. Gallic acid was used to generate a calibration curve. The results were then reported as gallic acid equivalents.

Bark hydrolysis

Bark was hydrolyzed in an aqueous solution of sulfuric acid. The bark was mixed with an acid solution and heated in oil bath, under reflux, with magnetic stirring. The temperature, reaction time, acid concentration, solid/liquid ratio and particle sizes were determined by the experimental design (**Error! Reference source not found.**). At the end of the reaction time, the mixture was cooled in a cooling 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 of bark

was completed to 1 L and was kept refrigerated for further analysis.

HPLC analyses

Separation and quantification of hydroxymethylfurfural and furfural in the bark hydrolysate were performed using a Waters system consisting of 600 Waters pump with 600 Waters controller, column thermostat, and UV detector at 280 nm. A C18 column was used as a stationary phase using isocratic conditions with water:methanol:acetic acid (80:10:3) as the eluent. All eluents were degassed before use by flushing helium through for 20 min. The samples were injected through a 20 µL full loop and separations were performed at 25 °C with a flow rate of 1 mL/min.

Screening experimental design methodology

In order to study and optimize the phenolic content in bark by polysaccharides hydrolysis, a 2^5 factorial fractional screening experimental design with two levels was used. The effects of five factors on the weight loss, holocellulose content, Klason lignin, simple sugar yield, furfural and hydroxymethylfurfural yield were studied. The real and experimental coded factors are shown in T**Error! Reference source not found.**. The aim of this approach was to identify the main factors that had effects on the responses and to validate the first-order model ((1). The model was composed of three terms: a constant term (β_0), a sum of the main effects for each factor, and a sum of the interaction effects. In the second term, β_i is the coefficient of the effect of the i factor, and x_i is the level of the i factor. In the third term, β_{ij} is the coefficient of the effect of the interaction between factors i and j. Some factors' effects were aliased with second-order interactions considered as negligible. The third-order interactions were also negligible. The experimental design is shown in Table 2.

$$y = \beta 0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i,j=1}^{k} \beta_{ij} x_i x_j \quad (1)$$

RESULTS AND DISCUSSION

Characterization of chemical bark composition

The chemical composition of Norway spruce bark is shown in Table 3. The major components in bark are phenolic compounds, mainly represented by lignin, tannins and carbohydrates – represented by cellulose and hemicelluloses. Inner bark has lower lignin content, compared to outer bark – respectively, 12% and 32.8%. Nevertheless, inner bark has more extractives than outer bark – respectively, 32.1% and 15.6%. The total phenolic content of each extractive fraction is presented in Table 4. The yield of total phenols in the toluene/ethanol extracts and water was 772.6 mg/g and 401.6 mg/g (gallic acid equivalent), respectively. Inner bark had a lower phenolic content in water extracts than outer bark – respectively, 275.5 mg/g and 436.1 mg/g. We can deduce that the bark extractives are composed of 51% phenolic compounds.

Table 1
Factors, real and coded level of fractional factorial experimental design

Factors	Level	
Coded level	-1	1
Temperature	80 °C	100 °C
Reaction time	5 hours	24 hours
Acid concentration	3%	10%
Solid/liquid ratio	1/5	1/10
Particle size	<420 µm	420<d<800 µm

Table 2
Experimental table and responses of fractional factorial experimental design

Run	Experimental factors					Responses				
	Temperature (X1)	Reaction time (X2)	Acid concentration (X3)	Solid/liquid ratio (X4)	Particle size (X5)	Mass loss	Klason lignin	Holocellulose	Total phenols	Furfural
1	-1	-1	-1	-1	1	0.2900	0.4303	0.4064	21.709	0.012
2	-1	-1	1	1	-1	0.2806	0.4376	0.5348	9.846	0.140
3	-1	1	-1	1	-1	0.2892	0.4095	0.5337	13.556	0.781
4	-1	1	1	-1	1	0.3490	0.6097	0.3869	14.604	1.199
5	1	-1	-1	1	1	0.2863	0.4331	0.5486	9.571	0.011
6	1	-1	1	-1	-1	0.3832	0.5012	0.4679	8.107	3.471
7	1	1	-1	-1	-1	0.3869	0.4738	0.4813	8.053	2.564
8	1	1	1	1	1	0.2690	0.6148	0.3727	4.0638	11.144
9	-1	-1	-1	-1	1	0.2890	0.4518	0.3917	21.088	0.012
10	-1	-1	1	1	-1	0.2875	0.4496	0.4884	8.931	0.372
11	-1	1	-1	1	-1	0.2662	0.3903	0.4843	14.289	0.442
12	-1	1	1	-1	1	0.3336	0.5885	0.3465	14.263	0.853
13	1	-1	-1	1	1	0.2685	0.4453	0.5125	8.366	0.014
14	1	-1	1	-1	-1	0.3926	0.5178	0.4987	6.883	2.890
15	1	1	-1	-1	-1	0.3833	0.4888	0.4790	8.319	2.894
16	1	1	1	1	1	0.2686	0.6261	0.3467	4.3974	15.120
17	-1	-1	-1	-1	1	0.2791	0.4411	0.4212	20.468	0.014
18	-1	-1	1	1	-1	0.3041	0.4379	0.4807	7.610	0.256
19	-1	1	-1	1	-1	0.2744	0.3929	0.4715	13.105	0.667
20	-1	1	1	-1	1	0.3522	0.6366	0.3667	13.314	1.019
21	1	-1	-1	1	1	0.2774	0.4722	0.5415	10.677	0.109
22	1	-1	1	-1	-1	0.3851	0.4910	0.5296	6.481	3.073
23	1	1	-1	-1	-1	0.3846	0.4717	0.4863	9.396	3.441
24	1	1	1	1	1	0.2682	0.6374	0.3207	13.132	13.132

Then, the separation between the inner and outer bark was difficult with low advantages and high cost. It will be important to keep this fraction in order to increase the phenolic content of bark and enhance the resins property.

Table 3
Chemical composition of spruce bark

	Extractives	Cellulose	Hemicellulose	Klason lignin
Whole bark	23.3%	29.0%	21.7%	24.5%
Inner bark	32.1%	25.4%	26.0%	12.0%
Outer bark	15.6%	32.8%	16.1%	32.8%

Table 4
Total phenolic content of spruce bark extracts

Solvent	Material	Total phenolics (mg/g of extract)
Toluene ethanol	Bark	772.6
	Inner bark	775.5
	Outer bark	758.8
Water	Bark	401.6
	Inner bark	436.1
	Outer bark	276.3

Table 5
Analysis of fractional factorial experimental design

Coefficients	Mass loss	Klason lignin	Holocellulose	Total phenolics	Furfural
β_0	0.31450	0.49371	0.45410	10.90951	2.65123
β_1	0.01495	0.02073	0.01136	-3.48910	2.17066
β_2	0.00425	0.03464	-0.03107	-0.73527	1.78680
β_3	0.00829	0.05197	-0.02574	-2.30689	1.73784
β_4	-0.03619	-0.01481	0.01557	-1.81417	0.86443
β_5	-0.02027	0.03853	-0.04059	1.36158	0.90194
β_{24}	-0.00693	0.00303	-0.01995	-0.19190	1.44051
β_{34}	-0.00997	-0.00169	-0.01702	0.66363	1.57864
R2	0.9804	0.9742	0.9193	0.9823	0.9794
R2 adjusted	0.9718	0.9639	0.8871	0.9760	0.9721
Residual standard error	8.080e-3	1.436e-2	2.388e-2	8.003e-1	7.734e-1
Fexp	11.86	86.47	26.05	126.66	108.46
Prob > F	6.370e-5	1.622e-11	1.320e-7	8.321e-13	2.79e-12

Screening experimental design analysis

The results of screening experimental design are presented in Table 2. The analysis of variance and the coefficients of the model are presented in Table 5. The screening experimental design chosen allows selecting the main factors

that influence the responses. The effects of five factors were analyzed on three responses. The considered responses were mass loss, Klason lignin content, and holocellulose content of spruce bark, the soluble phenolic compounds and the furfural yield in the hydrolysis solution.

The Pareto chart presents the effects of all the factors on the response. The light gray bar presents the effect value of the factors. The dark gray zone presents the limit between significant and negligible factors. The significant value was calculated from statistical student tests for a p-value of 5%. The curve presents the cumulative effects of the factors on the response. The factors are ranked from most to least significant. A factor with a negative effect has a higher value for the low level than the value for the high level.

The R-square value of the mass loss response was 0.9804, meaning that 98.04% of the total variation in the mass loss value can be attributed to the factors that were investigated. The total mass loss variation set between 21.5% and 39%. The R-square of Klason lignin content was 0.9742. The Klason lignin content set between 33.6% and 58.9%. The R-square of holocellulose content response was 0.9193. The minimum and maximum values of the holocellulose content were, respectively, 31.7% and 61.5%. The R-square of soluble phenolic content was 0.9823. The total phenolic content variation set between 21.1 mg/g (mg per g of dried bark in gallic acid equivalent) and 1.4 mg/g. The R-square of furfural response was 0.9794. The variation was comprised between 0 and 13.1 mg of furfural per gram of dried bark.

All responses variations are explained by the factors investigated.

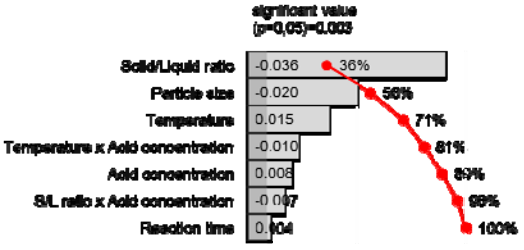

Figure 1: Pareto chart with factors influencing mass loss of hydrolyzed bark

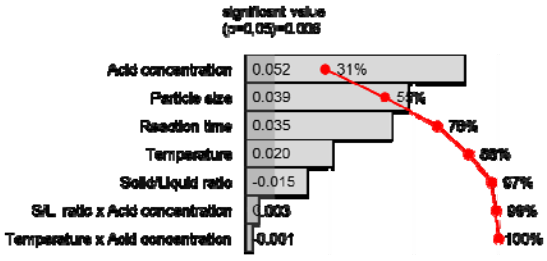

Figure 2: Pareto chart with factors influencing Klason lignin content of hydrolyzed bark

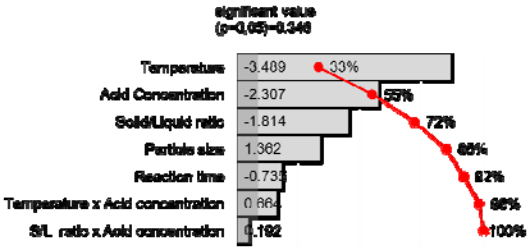

Figure 3: Pareto chart with factors influencing total phenolics in acid solution after bark hydrolysis

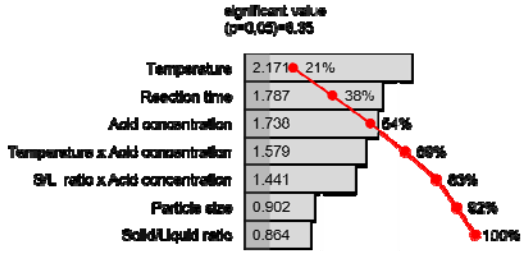

Figure 4: Pareto chart with factors influencing furfural in acid solution after bark hydrolysis

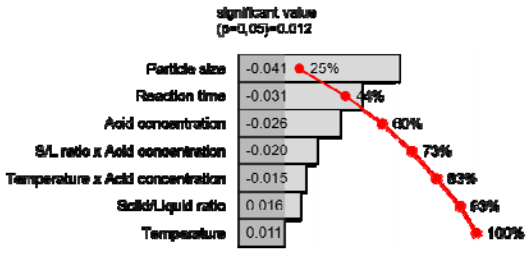

Figure 5: Pareto chart with factors influencing holocellulose content of hydrolyzed bark

Effect of temperature

Temperature had a significant impact on the mass loss (Figure 1) and Klason lignin content of hydrolyzed bark (Figure 2) with effects of 15 and 12%, respectively, of the response variation, and on the total phenolics (Figure 3) and furfural (Figure 4) contents of the acid solution after bark hydrolysis with, respectively, 33% and 21% of the response variation. Temperature effects were positive on the mass loss, Klason lignin content of bark and furfural content. The hydrolysis of bark and, probably, the extraction of soluble phenolic compounds were enhanced by high temperature. High temperature increased the degradation of polysaccharides and produced higher contents of degradation products, like furfural. Furfural is a degradation product of pentose from hemicelluloses. The temperature effect was negative on the total soluble phenolics. The total soluble phenolic compounds in the acid solution decreased when the temperature increased due to the degradation and condensation reactions of phenolics, such as tannins, lignin and furfural.[18,19]

Effect of reaction time

Reaction time had a significant effect on the Klason lignin content of hydrolyzed bark and holocellulose (Figure 5) and furfural contents with, respectively, 21%, 19% and 17% of the response variation. Reaction time had a negligible effect on the mass loss. Reaction time had a low effect on the total phenolics in solution. Nevertheless, this effect was not negligible. Reaction time effects were positive on Klason lignin content of bark and on furfural in solution. A reaction time of 24 hours increased the Klason lignin content of bark due to a better polysaccharides hydrolysis and soluble compounds extraction. The degradation of polysaccharides increased with a long reaction time. Reaction time effects were negative on the holocellulose content of bark due to the increase of holocellulose hydrolysis.

Effect of acid concentration

Acid concentration had significant effects on the Klason lignin and holocellulose contents of bark with, respectively, 31% and 16% of the total response variation, and also on the total phenolics and furfural in the acid solution with, respectively, 22% and 17% of the response variation. Acid concentration had a low effect on the mass loss. The effects were positive on the Klason lignin content of bark and on the furfural content of the acid solution after the reaction. An acid concentration of 10% increased Klason lignin content due to a better hydrolysis of poly-

saccharides, compared to the hydrolysis with an acid concentration of 3%. Acid concentration effects were negative on the holocellulose content of bark due to an increasing hydrolysis rate, and also on the soluble phenolics content of the acid solution after the reaction, due to the degradation or polymerization reaction of phenolic compounds.[20,21]

Effect of particle size

Particle size had a significant effect on all factors. The holocellulose content, Klason lignin content, and mass loss of bark were important with effects of 25, 24 and 20%, respectively, of the total response variation. Particle size effects were positive on the Klason lignin content of bark and soluble phenolic compounds in the acid solution after bark hydrolysis. Particle size effects were negative on the mass loss and holocellulose content of bark after hydrolysis. Small particles were more easily hydrolyzed and more soluble compound could be extracted from bark, compared to large particles, due to the diffusion of the solvent into the particles of bark. Normally the Klason lignin content increases with the hydrolysis rate, and the holocellulose content decreases with the hydrolysis rate. Then, the particle size effects should be negative on the Klason lignin content, because the hydrolysis rate of large particles is lower than hydrolysis rate of small particles. The particle size effect should be positive on the holocellulose content of hydrolyzed bark for the same reasons. This phenomenon could be explained by the impact of hydrolysis rate on the grinding of bark and by the impact of interactions, which were considered as negligible. A high hydrolysis rate gives smaller particles of bark after grinding, compared to a low hydrolysis rate. A factor aliased with an important interaction is biased by this interaction.

Effect of solid/liquid ratio

Solid/liquid ratio had a significant effect particularly on the mass loss and total phenolics in bark hydrolysate with, respectively, 36 and 17% of the response variation. The effects of solid/liquid ratio were less significant on the Klason lignin and holocellulose content of bark, and on the phenolics and furfural contents of the acid solution. A low solid/liquid ratio increased the mass loss, Klason lignin content and total phenolics of bark hydrolysate and decreased the holocellulose content of bark and furfural content of bark hydrolysate, due to the extraction and hydrolysis enhanced by more acidic solution, compared to a high solid/liquid ratio. Its effect on the Klason lignin yield and holocellulose content was less significant than on the mass loss and soluble phenolic compounds, which could be explained by the effect of the solid/liquid ratio on the solubilisation of the extractive content of bark. A low solid/liquid ratio enhanced the extraction of tannins and simple phenolic compounds. So, a high solid/liquid ratio would be preferred in order to enhance the polysaccharides degradation and to limit the phenolic compounds extraction.

Effects of interactions

The interactions that were not considered negligible were the following: solid/liquid ratio x acid concentration aliased with temperature x reaction time and temperature x acid concentration aliased with reaction time x solid/liquid ratio. These interactions had an important effect on the mass loss and on the furfural and holocellulose contents of

bark. These interactions must be considered in a further study.

First-order model validation

The model chosen for the screening experimental design was a first-order model. A new assay was realized and the experimental conditions were set to the center points (level 0) of each factor in order to verify the first-order hypothesis. The predicted mass loss for the center point was 33.5%. The measured mass loss for the center point was 32.6%, i.e. there was no significant difference between the predicted and the measured values. The first-order polynomial model was accepted for the experimental domain explored. The phenomena can be described by a first-order model probably due to the easy hydrolysis of hemicelluloses with a low severity of the experimental conditions, and the low hydrolysis of cellulose, despite the high severity of the experimental conditions. Some interactions were not studied, nevertheless the interactions that were investigated were not negligible and will be considered in a further study.

CONCLUSION

Cellulose hydrolysis was difficult under the conditions of our experimental design. The hemicelluloses were hydrolyzed and degradation products were formed, such as hydroxymethylfurfural from hexoses and furfural from pentose. All factors investigated had significant effects on the responses. The phenolic content of bark was optimized. Nevertheless, there were probably some condensation reactions among the degradation products of carbohydrates, tannins and lignin. The first-order polynomial model was accepted on the experimental domain investigated. It would be interesting to study bark hydrolysis with a large experimental domain. Hemicelluloses hydrolysis could be optimized under lighter conditions than the conditions investigated in this experimental design. Harsher conditions are required to hydrolyze cellulose.

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