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Microwave-assisted extraction of lignin from triticale straw: Optimization and microwave effects

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ABSTRACT

Presently lignin is used as fuel but recent interests in biomaterials encourage the use of this polymer as a renewable feedstock in manufacturing. The present study was undertaken to explore the potential applicability of microwaves to isolate lignin from agricultural residues. A central composite design (CCD) was used to optimize the processing conditions for the microwave (MW)-assisted extraction of lignin from triticale straw. Maximal lignin yield (91%) was found when using 92% EtOH, 0.64 N H₂SO₄, and 148 °C. The yield and chemical structure of MW-extracted lignin were compared to those of lignin extracted with conventional heating. Under similar conditions, MW irradiation led to higher lignin yields, lignins of lower sugar content, and lignins of smaller molecular weights. Except for these differences the lignins resulting from both types of heating exhibited comparable chemical structures. The present findings should provide a clean source of lignin for potential testing in manufacturing of biomaterials.

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1. Introduction

Lignin is the second most abundant natural polymer after cellulose (Meister, 1996). It is a heterogeneous organic polymer made from aromatic units, and composed of different kinds of phenylpropanoid units bonded together by ether linkages or carboncarbon bonds. Despite the interest that it arouses as a starting material for the production of bioproducts and biomaterials (Lora, 2008; Gandini and Belgacem, 2008), lignin is presently mainly used as a fuel (26.5 kJ/g) (Meister, 1996). Lignin recovery from agricultural residues can create new opportunities for the production of aromatic biochemicals and biomaterials from plants. In an era where available fossil oil reserves are declining, renewable feedstocks such as agricultural and forestry lignocellulosic residues will become more and more valuable.

Currently, the main industrial methods used for extracting lignin from woody or non woody lignocellulosic feedstocks are the Kraft, soda, sulfite, and organosolv processes (Gierer, 1985; Pye and Lora, 1991). Most of these methods require high temperatures and high pressure. The high temperatures requested are usually reached by conductive heating from an external heat source, which implies reaction times of several hours. These long reaction times are not desirable because of high energy consumption and multiple unwanted side reactions. In an effort to improve lignin isolation from lignocellulosic material, we considered using microwave irradiation as energy source. Microwave energy has been successfully applied in various chemistry fields including chemical analysis, organic synthesis, and homogeneous and heterogeneous catalysis (Kingston and Haswell, 1997; De la Hoz et al., 2005; Strauss and Varma, 2006; Betancourt et al., 2007; Kappe, 2008). In most cases, replacing classical heating by microwave irradiation led to spectacular accelerations, due to the efficient internal heating produced by the direct coupling of microwave energy with the molecules present in the reaction mixture as opposed to a slow and inefficient energy transfer when using classical heating (Kappe, 2008). In addition to the higher speed and efficiency, microwave irradiation also led to more specific and selective reactions.

Microwave energy has been recently explored as a potential technology to enhance the pretreatment of various lignocellulosic materials for the production of biofuels (Eskicioglu et al., 2007a,b; Taherzadeh and Karimi, 2008; Alvira et al., 2010; Shi et al., 2011; Jackowiak et al., 2011). It has more rarely been applied to the specific extraction of lignin, either as a pretreatment technique prior to enzymatic mild acidolysis (Zoia et al., 2008) or in combination with ionic liquids (Tan et al., 2009; Sun et al., 2009; Fu et al., 2010). The first application allowed improving the lignin purity but was devoted to the pretreatment step rather than the extraction one. The second application, although promising, still depends on expensive ionic liquid solvents.

In this study, we explored the potential application of microwave energy for the extraction of lignin from triticale straw. An organosolv process was selected based on the easier isolation of lignin from the extracting medium as opposed, for instance, to a

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soda process. Several variables including the ratio of ethanol to water, the acid concentration, the temperature and the reaction time were evaluated. A central composite design (CCD) model was used to optimize the processing conditions. The yield and chemical composition of the extracted lignin were compared to those of lignin extracted from triticale straw using conventional heating under similar conditions to determine whether microwaves played a specific role in the process.

2. Methods

2.1. Materials

Triticale (Triticosecale X) straw used in this study was provided by Agriculture and Agri-Food Canada, Lethbridge, AB, Canada. The straw of Ultima cultivar was cultivated in Southern Alberta, Canada, in 2007. It was ground with a FOSS Tecator Cyclotec sample mill (FOSS, Eden Prairie, MN) equipped with a 1 mm opening screen and kept at room temperature until use.

Ethanol 95% was from Commercial Alcohols, Brampton, ON, and deionized water was obtained with a Milli-Q Advantage A10 system (Millipore, Mississauga, ON). Sulfuric acid (95.5–98%) was from Anachemia Canada Inc., Montreal, QC. Tetrahydrofuran (THF, HPLC grade) and methanol (MeOH, HPLC grade) were from Fisher Scientific, Ottawa, ON. Anhydrous pyridine (99.8%), deuterated chloroform, *N*-hydroxynaphthalimide, chromium(III)acetylacetonate, 2-chloro-1,3,2-dioxaphospholane, 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, cellulose (powder, ~20 μ m), sugars (glucose, xylose, mannose, galactose, arabinose) and furans (2-furaldehyde, 5-hydroxymethylfurfural) were from Sigma Aldrich, Oakville, ON. Birch wood xylan was from Fluka, Oakville, ON and Kraft lignin Indulin AT was from MeadWestwaco, Charleston, SC.

2.2. Microwave-assisted experiments

All microwave experiments were conducted using a MARSXpress™ (CEM Corporation, Matthews, NC, USA) microwave reaction system.

The measurement of microwave absorbtivity by various pure components was carried out using a pressure sealed vessel equipped with fiber optic temperature and pressure probes by heating a given mass of compound at a specific power (800 W) and monitoring the bulk temperature.

The extraction optimization was conducted using a rotor with 40 75-mL Teflon pressure sealed vessels turning above an IR temperature sensor. Ground triticale straw (1.00 g) was introduced in each of eight tubes, together with 20 mL of a solution containing water, ethanol (27-95%), and concentrated sulfuric acid in various amounts (0-1 N). The eight tubes were screw capped, shaken, evenly placed in the rotor to allow temperature measurement by the IR sensor, and heated at a given temperature (83-167 °C) during 30 min unless otherwise mentioned. No stirring was applied during the heating. The temperature program used consisted of a fast heating step using a fixed maximum power (1600 W) followed by a plateau step during which power varied to maintain temperature at the target value. At the end of the reaction, samples were allowed to cool down to room temperature (~ 1 h). The solid residue (=cellulosic pulp) was isolated by filtration, washed with an aqueous ethanol solution (same concentration as for the reaction), dried in an oven at 60 °C, and its amount was estimated gravimetrically. Ethanol was evaporated from the remaining filtrate fraction using a rotoevaporator at 40 °C and under vacuum, and deionized water was added to the concentrated filtrate to precipitate lignin. Lignin was then vacuum-filtered, washed with deionized water, and dried at 60 °C. Each experiment was conducted in duplicate.

The effect of irradiation time on the yields of lignin was investigated by using one set of conditions (81% (v/v) EtOH; 0.5 N H₂SO₄; 125 °C) and changing the time spent at 125 °C from 0 to 30 min.

2.3. Extractions using conventional heating

For comparison, lignin was also extracted from triticale straw using the same extracting conditions (81% (v/v) EtOH; 0.5 N H₂SO₄; 125 °C) and conventional heating (CH). Straw (1.00 g) was introduced in 30 mL stainless steel reactors together with 20 mL of the selected extracting medium. The reactors were closed and heated at 125 °C in a GC oven. Reactors were manually shaken every 30 min. After selected increasing times the reaction samples were allowed to cool down in a cold water bath (~1 h) and treated similarly to the microwave-irradiated samples. Each experiment was conducted in duplicate.

2.4. Experimental design

Response surface methodology (RSM) was used to determine the optimal processing conditions for the MW-assisted extraction of lignin from triticale straw. The effect of three variables (ethanol fraction, acid concentration, temperature) was investigated using a central composite design (CCD) and five levels for each variable.

The selected variables (ethanol/water fraction (X_1) , acid concentration (X_2) , temperature (X_3)) were coded according to the following equation:

$$X_i = \chi_i(\Delta X_i) + X_0 \tag{1}$$

where X_i is the actual value of the independent variable, χ_i is the coded level of the independent variable, X_0 is the actual value of the independent variable at the center point, and ΔX_i is the step change value over a coded level of 1. Preliminary experiments were conducted to identify the independent variables and appropriate ranges to construct the CCD.

Analysis of the experimental design data and calculation of predicted responses were carried out using Design Expert software (version 7.1.6) to fit the second order regression model:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i \chi_i + \sum_{i=1}^{3} \beta_{ii} \chi_i^2 + \sum_{i<1} \sum \beta_{ij} \chi_i \chi_j$$
(2)

where Y is the response variable (lignin yield) and β_0 , β_i , β_{ii} , and β_{ij} are the intercept, linear, quadratic, and interaction model coefficients, respectively. Additional experiments were subsequently conducted to verify the validity of the statistical experimental design under specific conditions.

2.5. Analytical techniques

The ash content of triticale straw was determined by gravimetric analysis at 550 °C according to the National Renewable Energy Laboratory (NREL) procedure (Ehrman, 1994). Extractives were estimated in ethanol-toluene according to the ASTM procedure (ASTM, 1996).

Acid insoluble lignin, acid soluble lignin and carbohydrates were determined according to the NREL procedure but by conducting the first hydrolysis step (72% H_2SO_4) at room temperature (22 ± 2 °C) instead of 30 °C to limit the extent of xylose degradation into 2-furaldehyde and polymeric insoluble compounds (Sluiter et al., 2008). The second hydrolysis step (4% H_2SO_4) was conducted in an autoclave at 121 °C for 1 h. Acid soluble lignin was estimated spectrophotometrically at 205 nm (BECKMAN Du 640) using the hydrolyzate obtained after isolation of the insoluble lignin and assuming an extinction coefficient of 110 L g⁻¹ cm⁻¹ (Ehrman, 1996). Analysis of the acid hydrolyzate by HPLC allowed quantifying the carbohydrates present in the straw (Sluiter et al., 2008).

Table 1

Chemical co	omposition	of	Triticosecal	e.	Χ	straw.
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Fraction	% Dry straw
Ashes	8.87 ± 0.03
Extractives (ethanol/toluene, 70/30)	9.08 ± 0.17
Lignin	
Klason lignin	16.7 ± 0.14
Acid soluble lignin	3.17 ± 0.17
Glucan	35.88 ± 0.81
Xylan	22.51 ± 1.41
Galactan	0.70 ± 0.10
Arabinan	2.30 ± 0.06
Mannan	0.38 ± 0.03

Sugars (glucose, xylose, mannose, galactose, arabinose) were analyzed by HPLC using a ICS-3000 chromatographic system from Dionex Canada Ltd. (Oakville, ON) composed of a dual pump, a detector/chromatography module and an electrochemical detector (Au working electrode/Ag/AgCl reference electrode). Acid solutions were neutralized with CaCO₃ and centrifuged prior to analysis. The separation was completed on a CarboPac SA10 Analytical column (250 mm \times 4 mm ID) maintained at 35 °C. The injection volume was 25 µL. The mobile phase consisting of 1 mM KOH was run at 1.5 mL min⁻¹. Peaks were identified and quantified by comparison with standards.

2-Furaldehyde (furfural) and 5-hydroxymethylfurfural (HMF) were analyzed by HPLC using a Hewlett Packard HPLC series 1100 system equipped with a diode array detector (DAD) model G1315A. The separation was completed on a Gemini-NX C18 column (3 μ m, 4.6 mm \times 150 mm) from Phenomenex (Torrance, CA) maintained at 40 °C. The injection volume was 10 μ L. The mobile phase consisted of a methanol gradient (10–20% during 35 min then up to 60% for 25 min) in H₃PO₄ 0.027 N. Detection and quantification were performed at 280 nm using standard solutions prepared in deionized water. All solutions were adjusted to pH 2 with either HCl or NaOH and centrifuged prior to analysis.

Molecular weight distributions of the extracted lignins were determined by gel permeation chromatography (GPC) using a multi-detection system from Viscotek (Houston, TX) equipped with a GPCmax that included an integrated pump, an autosampler, and a degasser. The multiple detector platform included a PDA detector, a refractive index detector, a four capillary viscometer and a two angles laser light scattering detector. However, the light scattering detector was not equipped with fluorescence filters and was only used for qualitative monitoring. Molecular weights were determined using universal calibration with the PDA detector at 280 nm and the viscosity detector. The universal calibration curve was based on polystyrene standards from Polymer Laboratories (Amherst, MA) and Sigma-Aldrich using the molar masses determined by the manufacturer and the intrinsic viscosities measured by the apparatus. The software OmniSEC from Viscotek was used for calibration and data collection. Separation was performed in tetrahydrofuran (THF) by injecting 100 μL of 1.5–2 mg mL⁻¹ solutions of lignin in THF into thermostatically controlled SuperRes columns (35 °C; PAS-102. PAS-102.5, PAS-103L. each $300 \text{ mm} \times 8 \text{ mm}$; PolyAnalytik (London, ON)). The flow rate was of 1 mL min⁻¹.

Quantitative ³¹P NMR spectra of the extracted lignins were obtained using published procedures with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane as phosphitylating reagent and *N*-hydroxynaphthalimide as internal standard (Argyropoulos, 1994; Crestini and Argyropoulos, 1997). The ³¹P NMR spectra of the resulting mixtures were obtained by using a Bruker 500 MHz spectrometer equipped with a broadband inverse probe dedicated to ³¹P, ¹³C, and ¹H acquisition. An acquisition time of 0.2 s, a delay time of 5.00 s and a number of scan of 512 were used in each analysis. All chemical shifts are reported relative to the signal of the product of water with the phosphitylating reagent (132.2 ppm). The content of hydroxyl groups was obtained by integration of the following spectral regions: aliphatic hydroxyls (149.1–144.6 ppm), syringyl (S) phenolic hydroxyls (143.3–141.9 ppm), condensed phenolic units (difference between 144.3–141.3 ppm and 143.3–141.9 ppm as previously done by Cateto et al. (2008)), guaiacyl (G) phenolic hydroxyls (140.6–138.6 ppm), p-hydroxyphenyl (H) phenolic hydroxyls (138.4–137.2 ppm), carboxylic acids (135.3–134.4 ppm).

FT-IR spectra of pure powdered solids were obtained using a Bruker Tensor Series FT-IR Spectrometer in the Attenuated Total Reflectance (ATR) analysis mode. Carbohydrate impurities in lignin were quantified after acid hydrolysis as described above for the determination of carbohydrates in straw. Elemental analyses were carried out by Galbraith Laboratories Inc., Knoxville, TN.

3. Results and discussion

3.1. Triticale straw composition

Analysis of various components of the triticale straw using the above described procedures led to the chemical composition summarized in Table 1. The straw used in this study was typical of straws belonging to the *triticum* species with close to 9% ashes, mainly composed of silica, 9% extractives, around 62% carbohydrates, and 20% total lignin, on a dry basis. This composition agrees well with values recently reported for triticale straw from Canada (Pronyk and Mazza, 2011).

3.2. Microwaves absorbtivity of lignocellulosics components

In conventional heating (CH), heat transfers from the heating device to the sample in a rather slow and homogeneous manner so that all points within the reaction medium are more or less at the same temperature. In microwave (MW) heating, all components present in the reactor heat simultaneously at a rate that is governed by their microwave absorbtivity. Because of this rapid and heterogeneous heating process, localized hot spots can be observed in the matrix heated in a MW system (Kingston and Haswell, 1997). In order to better understand the behavior of a complex matrix such as straw during the microwave-assisted extraction, we measured the MW absorbtivity of pure commercial cellulose, xylan, and lignin by heating a same mass of compound at the same power and monitoring the bulk temperature with a ther-



Fig. 1. Temperature profiles generated by heating 10 g of commercial cellulose, xylan, or lignin with the MARSXpress at 800 W.

mocouple deepened in the solid (Fig. 1). The high temperature reached by cellulose and xylan in few minutes (reaction was settled to stop at 230 °C) compared to a milder ramp and lower plateau for lignin show that carbohydrates are better MW absorbers than lignin (Fig. 1). The preferential degradation of cellulose by MW irradiation in the presence of lignin has been previously suggested, but not verified, to explain the higher yields of sugars obtained after MW pretreatment of lignocellulosic wastes (Taherzadeh and Karimi, 2008). When heating a lignocellulosic material in a MW system, cellulose and hemicellulose will thus heat preferentially compared to lignin which represents an advantage if, like in the present study, ones objective is to extract or degrade cellulose while maintaining lignin as integral as possible.

3.3. Response surface modeling

3.3.1. Single-factor tests

The effect of three parameters (ethanol concentration, acid concentration, temperature) on the yield of lignin was estimated using a CCD modeling. Fixed parameters included the reaction time, the microwave power, the type of acid, and the number of samples treated. Reaction time was fixed at 30 min to avoid variation of results that may be due to incomplete reactions when applying shorter reaction times. Power was fixed at its maximal value (1600 W) to ensure that maximal temperatures could be reached in less than 5 min. Sulfuric acid was selected as the most efficient acid catalyst after comparing the yield of lignin extracted when using H_2SO_4 , HCl, and CH₃COOH under similar conditions (81% EtOH, 0.5 N, 125 °C). Eight tubes were used in each experiment with a ratio of solid to liquid of 1–20 to warrant a good dispersion of the solid in the suspension.

Preliminary single-factor tests were conducted to determine the variation window for each of the three factors to be optimized. The maximum temperature that could be used with the present micro-wave tubes without getting important ethanol leakage through the pressure release vent was 170 °C; a temperature range of 83–167 °C was thus selected for the optimization. Acid concentrations above 1 N did not improve the yield of lignin but had a strong neg-

ative effect on the yield of pulp remaining, so that optimization was conducted between 0 and 1 N. A range of 27–95% (v/v) was selected for the fraction of ethanol in the extracting medium after running preliminary tests at 150 °C, 0.8 N H₂SO₄, and 41–95% (v/v) ethanol.

3.3.2. Model fitting and statistical analysis

A total of twenty runs were performed to optimize the three parameters (ethanol fraction (X_1) , acid concentration (X_2) , and temperature (X_3)) in the current CCD. Coded and actual values of the three independent variables and experimental results for the yield of lignin are shown in Table 2. These experimental results were fitted to the second-order polynomial regression model (Eq. (2)). The regression coefficients (β_0 , β_i , β_{ii} , and β_{ij}) and the results of the statistical analysis of variance (ANOVA) are presented in Table 3. According to the ANOVA results, the model was significant with a *p*-value below 0.0001, and the lack of fit was not significant with a *p*-value of 0.0571. Ethanol fraction (X_1) , acid concentration (X_2) , temperature (X_3) , the interaction term X_1X_2 and the quadratic terms X_1^2 and X_2^2 were significant model terms to predict the extracted lignin with *p*-values <0.05. The statistical analysis of the model suggests a good fitting of the experimental data as supported by the high determination coefficient (R^2 : 0.978) and adjusted determination coefficient (Adj *R*²: 0.959).

3.3.3. Optimization of the operating conditions

Coefficients given in Table 3 were used to generate response surfaces for the yield of lignin (Fig. 2). Response curves were plotted as a function of the ethanol fraction and acid concentration while keeping the temperature constant and equal to 125 °C (Fig. 2a), as a function of temperature and ethanol fraction while keeping the acid concentration constant and equal to 0.5 N (Fig. 2b), or as a function of temperature and acid concentration while keeping the ethanol fraction constant and equal to 61% (Fig. 2c).

Fig. 2 and Table 3 show that ethanol fraction, acid concentration, and temperature all had a positive impact on the yield of lignin. The strongest effect was attributed to the ethanol fraction as confirmed by the largest value of the coefficient (β_1) and in agree-

Table 2

Central composite experimental design and experimental data for solid hydrolysis and extraction of lignin and furfurals.

Run	Independent variables			Experimental yields				
	Ethanol fraction (%) ^a X ₁	Acid concentration (N) X ₂	Temperature (°C) X ₃	Extracted lignin (%) ^b	Remaining pulp (%) ^b	Total sugars (%) ^b	Furfural + HMF ^c (%) ^b	
1	41 (-1) ^d	0.2 (-1)	100 (-1)	4.35	60.94	3.28	0.09	
2	81 (+1)	0.2 (-1)	100 (-1)	8.80	56.54	0.00	0.44	
3	41 (-1)	0.8 (+1)	100 (-1)	5.80	56.02	6.02	0.11	
4	81 (+1)	0.8 (+1)	100 (-1)	11.37	45.63	0.00	0.46	
5	41 (-1)	0.2 (-1)	150 (+1)	6.65	45.46	13.35	1.11	
6	81 (+1)	0.2 (-1)	150 (+1)	11.30	32.66	0.00	5.70	
7	41 (-1)	0.8 (+1)	150 (+1)	7.55	41.94	14.90	2.12	
8	81 (+1)	0.8 (+1)	150 (+1)	17.60	25.36	0.00	6.93	
9	27 (-1.68)	0.5 (0)	125 (0)	3.59	50.85	14.44	0.54	
10	95 (+1.68)	0.5 (0)	125 (0)	15.35	32.87	0.47	4.54	
11	61 (0)	0 (-1.68)	125 (0)	4.26	83.71	0.00	0.00	
12	61 (0)	1 (+1.68)	125 (0)	11.46	44.26	13.24	0.80	
13	61 (0)	0.5 (0)	83 (-1.68)	8.22	46.04	4.31	0.92	
14	61 (0)	0.5 (0)	167 (+1.68)	15.38	14.38	15.15	7.10	
15	61 (0)	0.5 (0)	125 (0)	12.36	41.25	9.43	1.04	
16	61 (0)	0.5 (0)	125 (0)	11.59	39.82	8.43	1.27	
17	61 (0)	0.5 (0)	125 (0)	11.28	40.33	8.73	1.29	
18	61 (0)	0.5 (0)	125 (0)	12.48	41.25	8.89	1.03	
19	61 (0)	0.5 (0)	125 (0)	11.92	39.01	8.19	1.27	
20	61 (0)	0.5 (0)	125 (0)	12.12	38.88	8.78	1.36	

^a Volume percentage.

^b Yield in weight percentage of dry triticale straw.

^c HMF = 5-Hydroxymethylfurfural.

^d Numbers in parentheses are coded values in the experimental design.

 Table 3

 Regression coefficients (in coded terms) and ANOVA results for the model.

Coefficient	Extracted lignin	
	Value	Standard error
β_0	11.97	0.32
Linear		
β_1	3.26	0.21
β_2	1.71	0.21
β_3	1.69	0.21
Quadratic		
β_{11}	-0.93	0.20
β ₂₂	-1.50	0.20
β_{33}	-0.28	0.20
Interaction		
β_{12}	0.82	0.27
β_{13}	0.59	0.27
β_{23}	0.40	0.27
<i>p</i> -Value, Prob. > <i>F</i>		
Model	<0.0001 (signific	ant)
Ethanol fraction	<0.0001	
Acid concentration	< 0.0001	
Temperature	< 0.0001	
Lack of fit	0.0571 (not sign	ificant)
R^2	0.978	
Adj R ²	0.959	

ment with the lack of lignin solubility in acid water. As a consequence, reducing the proportion of ethanol below 40% (v/v) decreased considerably the amount of lignin extracted. Acid was essential in this process to ensure the hydrolytic cleavage of the bindings between hemicellulose and lignin as well as the fragmentation of lignin into smaller fragments that favors its solubilization in the aqueous ethanol medium. Although autohydrolysis has been reported to occur at elevated temperatures as a consequence of acetic acid release from hemicellulose (Browning, 1967), yield of isolated lignin remained very modest when no acid was used. The optimum extraction conditions for lignin as determined by using the Design Expert software with no constraints on energy and reagents were determined to be 92% EtOH, 0.64 N H₂SO₄, and 148 °C. Under these conditions, the predicted yield of lignin was found to be 18.2% of the initial dry straw, which corresponds to 91% of the original lignin in the triticale straw (19.9%, Table 1). In order to confirm the prediction, an experiment was carried out using these optimized conditions. An isolated lignin yield of 17.9% (90% of the original lignin) was obtained, which is close to the predicted value and which demonstrates the good capacity of the RSM to predict lignin yield in this microwave process. This yield is very high compared to the yield previously reported for the extraction of lignin from wheat straw using an acid ethanol/ water organosolv process and conventional heating (Xu et al., 2006), however one should mention that the conditions used in the reported system (60% ethanol, 0.1% HCl, 85 °C for 4 h) were much milder than in the present optimized conditions.

Quantification of the insoluble cellulosic pulp remaining after lignin extraction showed that the three parameters investigated all had a negative impact on the yield of pulp (Table 2). Indeed, when using higher temperatures or more acid, hemicellulose and cellulose were transformed into sugars as confirmed by the sugar yields measured under harsher conditions (up to 15% of the initial weight of straw) (Table 2). In addition, a significant fraction of these sugars (up to 7% of initial weight of straw) was transformed into furfural or 5-hydroxymethylfurfural (HMF), the common dehydration products of pentoses and hexoses, respectively, under hot acidic conditions, when increasing the temperature at or above 150 °C (Table 2). Depending on what the objectives of the industrial process are, various conditions may thus be preferable. Lignin yields exceeding 90% can be obtained by combining high acidity, high temperature and high ethanol fraction, however, these conditions will have an economic drawback due to the high energy and high content of reagents employed and to the small amount of pulp coproduced. For the rest of the study, we used milder conditions (81% EtOH; 0.5 N H₂SO₄; 125 °C) that allowed reaching both lignin and cellulosic pulp in satisfying yields (>70%).

3.4. Comparison with conventional heating

To determine whether microwaves played an athermal role in the reactivity and selectivity of the process, we compared lignin yields and characteristics obtained using microwave irradiation (MW) and conventional heating (CH) under similar conditions: 81% EtOH; 0.5 N H₂SO₄; 125 °C. The yields of lignin and cellulosic pulp obtained from triticale straw using either MW irradiation or CH for this set of conditions and various times are presented in Fig. 3. Interestingly, when microwave irradiation was used the straw fractionation was almost complete at the end of the temperature rise, i.e. after 3.5 min. After 30 min of microwave heating at 125 °C, the measured lignin yield was 13.9%, which again compared well with the predicted value of 14.3% given by the RSM for these conditions. As seen in Fig. 3, replacing conventional heating by microwave irradiation led to (1) a faster fractionation of the straw, (2) higher yield of lignin (a maximum lignin yield of 11.5% was reached after 3 h of CH), and (3) lower yield of cellulosic pulp. The faster straw fractionation observed upon MW irradiation can be easily explained by the faster temperature increase induced by MW. The higher yields obtained for MW-lignin are likely due to a more extensive lignin depolymerisation and subsequent higher solubilization in the alcoholic medium. Higher yields of lignin have also been observed by Zoia et al. (2008) when combining a MW pretreatment with an enzymatic mild acidolytic process to extract lignin from wood. The lower yield of cellulosic pulp is in line with the tendency of the latter to heat preferentially when exposed to MW.

3.5. Characterization of extracted lignins

Lignin samples isolated after 30 min of MW heating (MW1-lignin) or 3 h of CH (CH-lignin) under similar conditions (81% EtOH, 0.5 N H₂SO₄, 125 °C) were characterized using FT-IR, elemental analyses, acid hydrolysis, GPC, and ³¹P NMR. Elemental analyses did not reveal significant differences between MW1-lignin and CH-lignin (Table 4). In contrast, acid hydrolysis of both lignins followed by quantification of sugars indicated a higher content of carbohydrate impurities in CH-lignin than in MW1-lignin (Table 4). Sugars from MW1-lignin consisted mainly of arabinose (65%) while in CH-lignin both xylose (43%) and arabinose (34%) represented important fractions of the carbohydrate impurities. Wheat straw lignin is known to contain arabinoxylans as the main carbohydrate impurities with lignin being linked with arabinose side chains of xylan by ether bonds (Sun et al., 1997). The majority of these ether linkages occur at the α -position of the propanoid chains of lignin as initially suggested by their possible cleavage under acidic conditions (Mc Donough, 1993; Cornu et al., 1994; Lawther et al., 1996) and later confirmed by NMR and xylanase-based fractionation (Barakat et al., 2007). Applying MW instead of CH decreased significantly the xylose content of the lignin but had almost no effect on the arabinose content. This indicates that the glycosidic bonds contained in lignin carbohydrates are more sensitive to the type of heating than the benzyl ether linkages occurring between lignin and arabinose. The above noted higher MW absorbtivity of carbohydrates compared to lignin might explain the preferential heating and degradation of glycosidic linkages.



Fig. 2. Response surfaces of predicted lignin yield versus (a) acid concentration and ethanol fraction at a temperature of 125 °C; (b) temperature and ethanol fraction at an acid concentration of 0.5 N; and (c) temperature and acid concentration at an ethanol fraction of 61%.



Fig. 3. Lignin and cellulosic pulp collected after conventional heating (CH) or microwave irradiation (MW) (EtOH/H₂O: 81/19; 0.5 N H₂SO₄; 125 °C).

 Table 4

 Elemental analysis and sugar content of ligning extracted from triticale straw.

		-					
Lignin sample	Elemer	Elemental analysis (% w/w)					
	С	Н	Ν	0	S	content	
Triticale straw	42.54	5.93	0.70	43.50	0.13	61.77	
CH-lignin (125 °C; 81%	62.76	6.63	1.08	28.14	0.45	1.55	
EtOH; 0.5 N H ₂ SO ₄ ; 3 h)							
MW1-lignin (125 °C; 81%	61.38	6.57	1.17	29.23	0.32	0.69	
EtOH; 0.5 N H_2SO_4 ;							
30 min)							
MW2-lignin (148 °C; 92%	65.35	6.35	<0.5	26.94	0.54	0.39	
EtOH; 0.64 N H ₂ SO ₄ ;							
30 min)							

FT-IR analyses showed spectra characteristic of lignin spectra (aliphatic and aromatic O–H stretch ($3600-3000 \text{ cm}^{-1}$), C–H stretch in methyl and methylene groups ($3000 \text{ and } 2800 \text{ cm}^{-1}$), unconjugated C=O stretch ($1732-1707 \text{ cm}^{-1}$), aromatic skeletal vibrations (1595-1603, 1512, 1426 cm^{-1}), C–H deformation in methyl and methylene groups (1460 cm^{-1}), C–H deformation is ster groups typical of hydroxyphenyl/guaiacyl/syringil-type (HGS) lignins (1165 cm^{-1}), aromatic C–H deformation of syringyl (S) groups ($1115-1118 \text{ cm}^{-1}$) and C–O(H) deformation in primary alcohols (1030 cm^{-1})) (Faix, 1992). No significant difference was detected between the lignins extracted using MW or CH, thus suggesting little effect of the heating mode on the distribution of functional groups in the lignins.

The concentration of aliphatic, phenolic, and carboxylic OH units was determined in the two lignins (MW1-lignin and CH-lignin) by quantitative ³¹P NMR analysis after phosphitylation with 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane (Fig. S1; Table 5). In agreement with the FT-IR data, the two NMR spectra were rather similar except for an excess of aliphatic groups in the CH-

Table 6

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Sample	$M_{ m w}$ (g mol ⁻¹)	$M_{ m n}$ (g mol ⁻¹)	$\frac{M_{ m w}}{M_{ m n}}$
CH-lignin (125 °C; 81% EtOH; 0.5 N H ₂ SO ₄ ; 3 h)	8667	804	10.78
MW1-lignin (125 °C; 81% EtOH; 0.5 N H ₂ SO ₄ ; 30 min)	3166	734	4.31
MW2-lignin (148 °C; 92% EtOH; 0.64 N H ₂ SO ₄ ; 30 min)	1781	678	2.63

lignin and a stronger signal at 136.4 ppm for the MW1-lignin. The difference in content of aliphatic OHs in the two lignins was mainly due to the presence of four distinct signals at 145.7, 145.9, 146.6 and 146.8 ppm in CH-lignin that were not present in MW1-lignin (Fig. S1). Given the similarity of these chemical shifts with those that we measured for xylose in pyridine/CDCl₃ (145.8, 146.5, 146.8 and 147.2 ppm) and the results of sugar analyses, these signals were attributed to xylan fragments that remained attached to the lignin when using CH. As for the signal at 136.4 ppm, our attempts to compare it with signals of various phenolic and inorganic references remained unsuccessful. This signal was previously observed in milled lignin extracted from wheat straw (Crestini and Argyropoulos, 1997) thus indicating that it is inherent to the biomass (wheat or triticale) but not related to the use of ethanol or sulfuric acid. The three H. G. and S phenolic signals were observed in the two samples in proportions of approximately 20%. 45%, and 35%, respectively, regardless of the type of heating. The two types of heating also led to the same contents of aromatic (downfield signal) and aliphatic (upfield signal) COOH groups.

Molecular weights of MW1-lignin and CH-lignin were determined by GPC with polystyrene standards and THF as eluent (Table 6). While both lignins contained similar small fragments (100–400 g mol⁻¹), they differed in the content of larger molecular weight compounds. Indeed, CH-lignin contained polymers of molecular weights above 10,000 that were almost inexistent in the MW-lignin. As a result, the lignin obtained using CH was much more heterogeneous in molecular masses ($M_w/M_n = 10.8$) than the MW-lignin ($M_w/M_n = 4.3$). Microwave treatment thus degraded the macromolecular structure of lignin (or its sugars intra-links) to smaller units compared to conventional heating and allowed reaching a more homogeneous material in terms of molecular size.

The lignin extracted under optimized conditions (92% EtOH, 0.64 N H₂SO₄, and 148 °C), MW2-lignin, was also characterized using the same techniques (Tables 4–6). Elemental analysis showed a significantly less oxidized form for this lignin compared to the two lignins extracted under milder conditions (125 °C, 0.5 N) (Table 4). ³¹P NMR confirmed the trend and indicated that most of the missing oxygen was attributable to a loss of aliphatic OH (Table 5). The latter was most likely due to dehydration of the OH groups bound to the propanoid chains under the more drastic conditions. In addition, using higher temperature and acid concentra-

Table 5

Phenolic, aliphatic, and carboxylic hydroxyl groups present in MW-lignins and CH-lignin.

Sample	OH (mmol g ⁻¹)							
	Total COOH	H ^a phenolic	G ^a phenolic	S ^a phenolic	Condensed phenolic	Total phenolic	Total aliphatic	
CH-lignin (125 °C; 81% EtOH; 0.5 N H ₂ SO ₄ ; 3 h)	0.20	0.40	0.96	0.65	0.12	2.13	2.58	
MW1-lignin (125 °C; 81% EtOH; 0.5 N H ₂ SO ₄ ; 30 min)	0.22	0.52	1.06	0.85	0.18	2.61	1.60	
MW2-lignin (148 °C; 92% EtOH; 0.64 N H ₂ SO ₄ ; 30 min)	0.13	0.41	1.10	0.88	0.31	2.70	0.62	

^a H: Hydroxyphenyl; G: Guaiacyl; S: Syringyl.

tion also led to a lignin of smaller molecular weight (Table 6). The lignin extracted under optimized conditions thus appeared as a lignin of small molecular weight and containing little aliphatic OH, two favorable criteria for its application in blends with polyolefins.

4. Conclusions

A central composite design (CCD) was successfully used to determine the optimal processing conditions for lignin extraction from triticale straw. Maximal yield of lignin (91% of the total lignin) was predicted and experimentally confirmed. When compared to conventional heating, MW irradiation led to higher lignin yields, lower content of sugars associated to lignin, and lignins of smaller molecular weights. Except for these differences the lignins resulting from both types of heating exhibited comparable chemical structures. These findings demonstrate the potential applicability of MW to generate lignins of high purity and low molecular weight that can be applied as macromonomer in the production of biomaterials such as resins or foams.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.11.079.

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