Rapid Dissolution of Lignocellulosic Plant Materials in an Ionic Liquid

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Concerns regarding the non-renewable nature of, and pollution from, petroleum derived energy and commercial products has led to the concept of a biomass economy. As part of this vision for a society based on sustainable biomaterials, proposed biorefineries need to tackle the challenges of taking a wide diversity of raw biomass and rapidly and effectively transforming it into functionalizable platform molecules that can be derivatized into industrial and consumer products, or converted into biofuels (*1*). A substantial research effort is underway focussed on degrading biomass into smaller constituents using a variety of physical, chemical, and biological processes. One promising technology for the solubilization of biomass is ionic liquids (ILs), which has received considerable attention as a medium for efficient solubilization of a variety of materials. ILs also allow fractional separation when combined with solvent extraction (conventional, and green technologies such as supercritical CO_2), precipitation, and adsorption/absorption methods, and to conduct a wide range of chemical reactions using thermal, electrochemical, photochemical, and biocatalytic processes (*2*). As a potential pretreatment

technology for the biorefineries of the future, we report herein the first rapid dissolution of a range of coniferous and deciduous woods and grassy lignocellulosic plant materials in an IL using microwave radiation.

We chose for this proof-of-principle study the well-known IL 1-*n*-butyl-3-methylimidazolium chloride ([C_4MIM]Cl) [Fig. 1A], which has previously been shown to liquefy isolated biomass biopolymers (e.g., cellulose and lignin (*3*)) under both infrared and microwave radiation, to liquefy wheat straw under microwave radiation (4), and to partially liquefy wood under infrared radiation (*5*). Only one previous study (*6*) has completely liquefied wood using an IL (3,3'-ethane-1,2-diylbis(1-methyl-1*H*-imidazol-3-ium)), but extended reaction times at elevated temperatures (25 min at 120°C) were required and the principle was demonstrated for only a single conifer species (redwood). The six biomass materials studied herein (oak, ponderosa pine, smooth sumac, grape stem, flax shives, and triticale straw) were chosen based on their potential to represent various aspects of a biomass economy, and a range of lignocellulosic compositions.

Dissolution behaviour for all materials was similar, with rapid initial size reduction of the biomass over the first 15 to 30 s of irradiation. The remaining recalcitrant material after this initial treatment period required additional microwave exposure for about 30 to 60s to complete liquefaction (Fig. 1B-E). Upon cooling the fully liquefied solution to room temperature, no precipitation of dissolved components was observed, and the material remained as a dark brown, viscous mass. Dissolution limits for the six biomass types were approximately 5 %, while remaining as a relatively non-viscous liquid.

Worth noting is our finding that unprocessed biomass can be rapidly and completely dissolved via microwave irradiation in an IL after only brief drying (70°C under vacuum for 2 h) and grinding (1 mm mesh size), suggesting promise for this technology to be placed at the 'front end' of a biorefinery. Concerns have been expressed about the hygroscopic nature of ILs such as $[C_4MIM]Cl$ (*3*), potentially limiting their practical use for large-scale biomass dissolution without the presence of drying reagents in the reaction vessel itself. Many of the perceived limitations are due to the reported low solubility of celluloses in ILs containing small quantities of water and other protic or polar solvents (*3*,*5*). However, our biomass materials contained between 2 to 5 wt. % water prior to experimentation, and we observed no decrease in solubility relative to dissolution experiments performed on materials that were dried for 2 h at 105°C immediately before use and did not contain any residual free water content. At our biomass solubility limits (ca. 5 wt. %), the composite solution at complete dissolution would have contained between 0.1 and 0.3 wt. % water. These values place lower limits for the amounts of water that can be present in ILs, and still allow for the complete microwave-assisted dissolution of the biomass.

Analysis of the liquefied solution by liquid chromatography (LC) with refractive index (RI) and diode array/fluorescence detection (DAD/F) indicated varying degrees of structural decomposition. The polyphenolic lignin materials were not substantially decomposed to their constituent monomers (<10% of mass balance). Cellulosic and hemicellulosic degradation was similar, from 4 to 8% conversion to monomeric sugars, sugar alcohols, and associated degradation products. Large polymeric peaks were evident by LC-DAD/F, indicating the partial degradation of the original lignin biopolymers. Hexane extraction and GC-MS analysis of the

biomass-IL liquors did not reveal any significant compounds other than long-chain fatty acids and waxes, attesting to the residual polymeric integrity and high polarities of the lignins, and negligible partition coefficients for degradation products into a nonpolar extractant. We note the potential to control the degradation process during dissolution by tuning the solvation via IL identity, type of atmosphere (oxidizing or inert), pressure, solvent modifiers, and catalysts, among others. The results suggest that all types of lignocellulosic biomass may be liquified using microwave irradiation in ILs, allowing for further work to better guide the proceess and achieve the desired platform molecules.

References and Notes

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Acknowledgements

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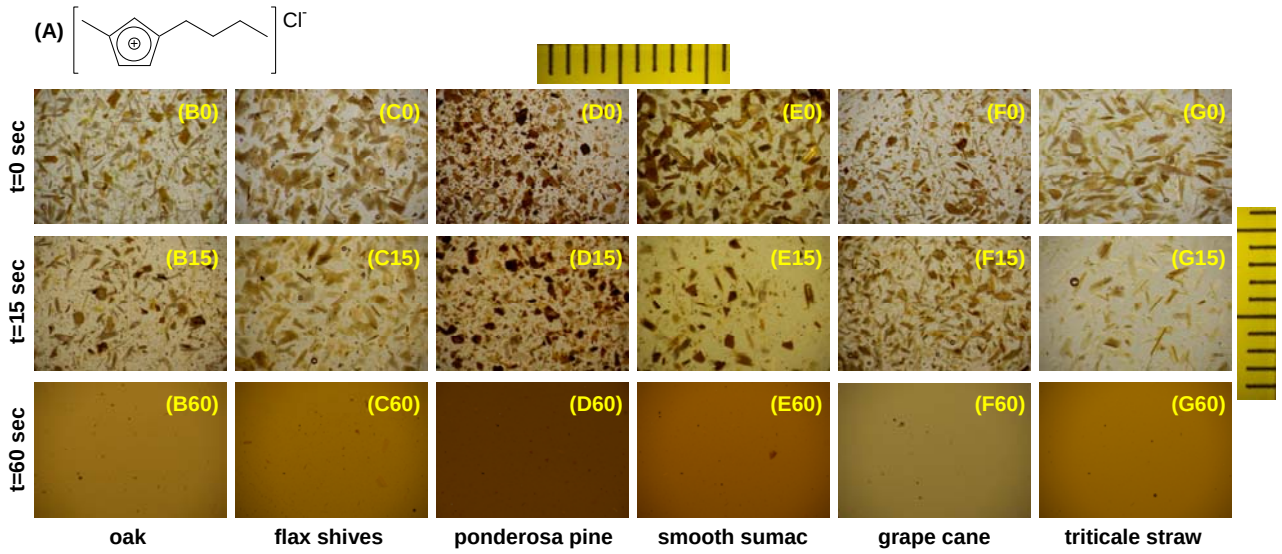


Fig. 1. (**A**) Structure of [BMIM]Cl. (**B**, **C**, **D**, **E**, **F**, and **G**) Dissecting microscopic images (4× magnification) of the (**B**) oak wood, (**C**) flax shives, (**D**), ponderosa pine wood, (**E**) smooth sumac wood, (**F**) grape cane, and (**G**) triticale straw. Liquefaction processes in [BMIM]Cl at 0 (upper row), 15 (middle row), and 60 seconds (lower row) microwave irradiation. Scale gradations are 1 mm.

Supporting Online Materials

1. Materials and Methods

1.1. Materials

1.1.1. Commercial Chemicals

The ionic liquid 1-*n*-butyl-3-methylimidazolium chloride ([C_4MIM]Cl) was purchased from Sigma-Aldrich, dried in a vacuum oven (24" Hg) at 70ºC for 24 h, and stored in a dessicator at room temperature before use. Calcium carbonate was purchased from Sigma-Aldrich and used as received. Sulfuric acid, phosphoric acid, methanol, and hexane were purchased from Caledon Laboratories. All chemicals were of reagent grade, with the exception of HPLC grade methanol used for liquid chromatography (LC) analysis. Water used for LC analysis was Millipore Milli-Q grade; all other water used for dilutions was distilled quality.

1.1.2. Biomass Samples

Unless stated otherwise, all biomass samples were cut into 5 to 10 cm lengths, dried at 70°C in a vacuum oven for 2 h, ground with a Wiley mill using a 0.35 mm blade gap and a 1 mm screen, and stored either in a freezer at -30°C until use.

White oak (*Quercus alba* L.) and ponderosa pine (*Pinus ponderosa*) branches were obtained from cuttings during May 2007 near Summerland, British Columbia, Canada. Smooth sumac (*Rhus glabra*) main stems were obtained from cuttings during March 2007 near Summerland, British Columbia, Canada. Grape cane (*Vitis vinifera* cv. Pinot Noir) was obtained from prunings during May 2007 near Penticton, British Columbia, Canada.

Flax shive (*Linum usitatissimum*) was obtained from Biolin Research Inc. (Saskatoon, Canada). It was ground with a Wiley mill using a 0.35 mm blade gap and a 4 mm screen. Bulk ground flax shive was screened using 8 mesh (2 mm opening) and 18 mesh (1 mm opening) screens. The screened flax shive with a particle size between 1 and 2 mm was further separated by air flotation to remove residual fiber.

Triticale straw (*Triticosecale Witt.*) was obtained from Lethbridge, Alberta, Canada.

1.2. Biomass Dissolution

Samples of biomass (0.1 g for 5 wt. % solutions) were placed in 3 mL glass Reacti-Vials, [C_4MIM]Cl was added (1.9 g), and the vials sealed with teflon rubber laminated discs and vortexed for 10 s to ensure complete mixing of biomass and solvent. Vials were then microwaved using a household unit (Danby Model DMW606W; 700 W operating with magnetron at 2450 MHz) with sequential 5 s microwave pulses and 5 s of vortexing, for 60 s total irradiation times.

Following dissolution, 0.5 g subsamples of solution were transferred to a small test tube, diluted 10:1 (v/w) with 4% sulfuric acid to prevent condensation of dissolved cellulosic components and their subsequent precipitation, and vacuum filtered through Whatman no. 1 qualitative filters (42.5 mm diameter; 11 μm nominal pore size). Direct filtration under vacuum of the undiluted solution was not possible due to its high viscosity.

1.3. Imaging and Post-Processing

Samples were collected from the Reacti-Vials at 0 s, 15 s, and 60 s microwave irradiation time intervals using the outside of glass pasteur pipette tip (to ensure unbiased sampling of liquid and particulates) and placed on a glass microscope well slide with 1 cm well-stickers at a liquid depth of 0.5 mm. Imaging was performed at 4× and 15× magnification using an Olympus SZX12 stereomicroscope with a Nikon DZX digital camera. Images were color white balanced.

1.4. Analysis

1.4.1. Liquid Chromatography with Refractive Index Detection (LC-RI)

Liquified biomass samples (5 wt. % in $[C_4MIM]Cl$) were allowed to cool to room temperature, diluted 10:1 (v/v) in water, and filtered through a 0.45 μm polyvinylidene difluoride (PVDF) membrane filter. Analysis of carbohydrates was conducted on an Agilent 1100 HPLC system with a refractive index detector. The LC column was a Phenomenex Rezex RCM-Monosaccharide column (300 mm × 7.8 mm × 8 μm) with Millipore grade water as the eluent (0.6 mL/min). The column and detector were maintained at 80°C with an injection volume of 50 μL. Identification and quantitation of compounds was performed with authentic samples of furfural, L-lactic acid, D-cellobiose, trehalose, sucrose, maltose, D-(+)-glucose, succinic acid, D-(+)-xylose, D-(+)-galactose, D-(+)-mannose, L-(+)-arabinose, acetic acid, ribitol, erythritol, glycerol, ethanol, lactic acid, arabitol, galactitol, xylitol, sorbitol, and 5-hydroxy-2-furaldehyde. Coelution of the $[C_4MIM]Cl$ solvent peak with furfural, L-lactic acid, D-cellobiose, trehalose, sucrose, and maltose prevented reliable identification and quantitation of these compounds.

1.4.2. Liquid Chromatography with Diode Array/Fluorescence Detection (LC-DAD)

Liquified biomass samples (5 wt. % in $[C_4MIM]Cl$) were allowed to cool to room temperature, diluted 4:1 (v/v) in 4% sulfuric acid, and filtered through a 0.45 μm PVDF membrane filter. Analysis of phenolic compounds was conducted on an Agilent 1100 HPLC system with a diode array detector. The LC column was a Phenomenex Luna C_{18} column (150 mm × 3.0 mm × 5 μm) with a Phenomenex Inertsil 5 ODS C_{18} guard column (30 mm × 4.6 mm × 5 μm). A gradient solvent system was used with 50 mM phosphoric acid (solvent A) and methanol (solvent B). The elution profile had the following proportions (v/v) of solvent B: 0 min, 10%; 0 to 30 min, 10% to 25%; 30 to 50 min, 25% to 45%; 50 to 55 min, 45% to 100%; 55 to 60 min, 100%; and 60 to 65 min, 100% to 10%. The solvent flow rate was 1.0 mL/ min. UV-Vis spectra were collected between 200 nm and 800 nm at 1 s intervals over the course of the chromatographic run. Absorbance channels at 220 nm, 280 nm, 320 nm, 360 nm, and 525 nm were continuously monitored. Fluorescence detection was continuously monitored at 348 nm using an excitation wavelength of 280 nm.

Total phenolic concentrations in the biomass extracts were estimated by integrating non-solvent areas in the chromatograms at 280 nm using chlorogenic acid as a quantification standard. Percent conversion of total polymeric lignins to soluble phenolics was calculated by dividing the calculated mass of total phenols in solution determined by LC-DAD by the total acid-soluble and acid-insoluble lignin determined on the starting materials (see section 1.4.5).

1.4.4. Gas Chromatography with Mass Spectrometric Detection (GC-MS)

Liquified biomass samples (5 wt. % in [C_4MIM]Cl) were allowed to cool to room temperature, extracted 1:1 (v/v) with hexane using gentle vortexing for 30 s, and filtered through a 0.45 μm PVDF membrane. GC-MS analysis was conducted on an Agilent 6890 gas chromatograph with an Agilent 5953 mass selective detector (MSD). The injection volume was 1 μL, and the injector temperature was held at 220ºC for the duration of the run and operated in the split mode at a 10:1 split ratio. Ultrahigh-purity helium was the carrier gas at a constant flow rate of 1.2 mL/min. A DB-5 capillary column (0.32 mm × 30 m × 0.25 μm) was used with the following temperature program: 50ºC, hold 10 min; 10ºC/min to 280ºC; and hold at 280ºC for 27 min to give a total run time of 60 min. The MSD transfer line, quadrupole, and source were maintained at 300ºC, 150ºC, and 230ºC, respectively. The MSD was operated in the full scan mode without solvent delay at an ionization energy of 70 eV over the m/z range from 50 to 650.

1.4.5 Carbohydrates, Lignin, Extractives, Ash, and Moisture Analyses of Starting Biomass

Determination of structural carbohydrates, lignin, extractives, ash, and moisture contents of the starting biomass materials followed standard analytical methods established by the National Renewable Energy Laboratory (NREL) (*1-5*). LC-RI conditions for the determination of structural carbohydrates under these standard methods were as given above in section 1.4.1.

2. Supporting Tables

Table S1. Carbohydrates, lignin, extractives, ash, and moisture contents of the oak, ponderosa pine, smooth sumac, grape cane, flax shives, and triticale straw starting materials. Values are the weight percent mean±range of replicate analyses.

	Carbohydrates				Lignin				
Material	**Glucose**	**Xylose/ Galactose**	**Mannose/ Rhamnose**	**Arabinose**	**Acid-soluble**	**Acid-insoluble**	**Extractives**	**Ash**	**Water**
Oak wood	31.2±0.2	21.9±1.6	nd[a]	0.9±0.2	8.1±0.3	23.9±0.6	6.9±0.4	4.5±0.0	2.6±0.3
Ponderosa pine wood	22.3±2.6	20.9±2.2	nd	2.1±0.3	5.2±0.1	47.7±0.5	1.3±0.1	2.9±0.1	2.0±0.1
Smooth sumac wood	18.7±1.1	17.4±0.1	nd	8.9±0.0	4.6±0.2	29.1±0.7	13.1±0.2	7.3±0.1	2.7±0.1
Grape cane	31.1±0.6	18.0±0.7	nd	nd	5.7±0.1	26.2±0.8	15.2±1.1	3.4±0.0	2.1±0.0
Flax shive	35.0±0.3	23.7±0.5	nd	nd	5.8±0.1	18.9±0.5	15.2±1.4	2.6±0.1	1.4±0.2
Triticale straw	29.7±1.4	21.7±0.3	nd	nd	8.1±0.0	28.1±0.4	7.2±1.9	3.8±0.0	2.5±0.3

[a] nd=not detectable.

Table S2. Percent conversion of polymeric cellulosic and hemicellulosic components to monomeric carbohydrates, carbohydrate degradation products, sugar alcohols, and organic acids after 60 s irradiation. Values are the conversion percent mean±range of replicate analyses.

	Oak	**Ponderosa pine**	**Smooth sumac**	**Grape cane**	**Flax shive**	**Triticale straw**
Glucose	0.5±0.1	0.6±0.1	1.3±0.1	0.5±0.1	0.6±0.2	0.6±0.1
Succinic acid	nd[a]	nd	nd	nd	nd	nd
Xylose/Galactose	3.0±0.2	0.8±0.1	3.5±0.5	3.4±0.1	3.4±0.4	8.4±0.3
Mannose/Rhamnose	nd	nd	nd	nd	nd	nd
Arabinose	58.7±9.2	27.9±4.3	6.7±1.2	nd	nd	nd
Acetic acid[b]	nd	nd	nd	8.2±0.6	5.5±1.1	7.1±0.3
Ribitol	nd	nd	nd	nd	nd	nd
Erythritol	nd	nd	nd	nd	nd	nd
Glycerol[b]	1.2%	nd	0.5±0.1	2.8±0.2	0.4±0.0	1.4±0.1
Ethanol	nd	nd	nd	nd	nd	nd
Lactic acid	nd	nd	nd	nd	nd	nd
Arabitol	nd	nd	nd	nd	nd	nd
Galactitol	5.7±0.3	6.8±0.2	10.7±0.8	7.7±1.2	5.1±1.0	5.4±1.0
Xylitol	nd	nd	nd	nd	nd	nd
Sorbitol	nd	nd	nd	nd	nd	nd
5-Hydroxy-2-furaldehyde[b]	0.7±0.1	0.6±0.1	5.6±0.3	0.7±0.1	0.8±0.0	0.6±0.0

[a] nd=not detectable. [b] Analytes were assumed to arise from cellulose for percent conversion calculations.

Table S3. Percent conversion of total polymeric lignins to soluble phenolics (chlorogenic acid chromophore equivalents) after 60 s irradiation. Values are the conversion percent mean±range of replicate analyses.

Material	Conversion
Oak wood	26.2±0.7%
Ponderosa pine wood	36.3±1.0%
Smooth sumac wood	34.4±0.8%
Grape cane	20.5±0.5%
Flax shive	125.1±1.4%
Triticale straw	44.6±0.5%

3. Supporting Figures

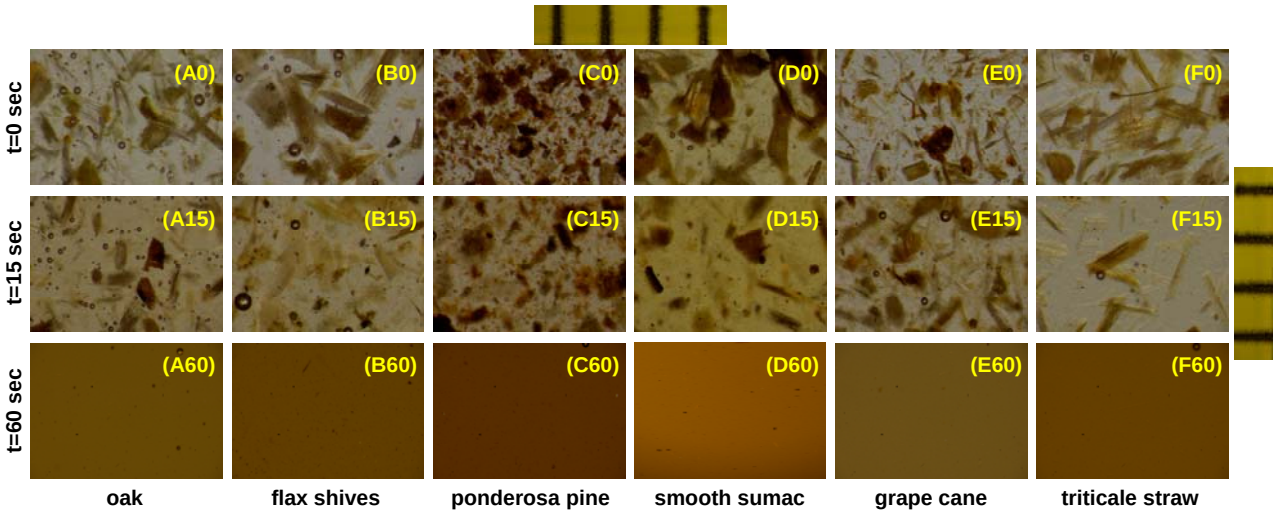
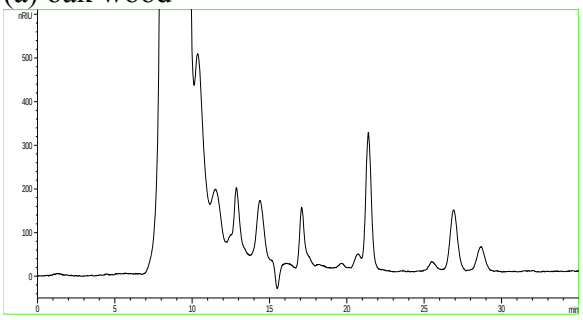
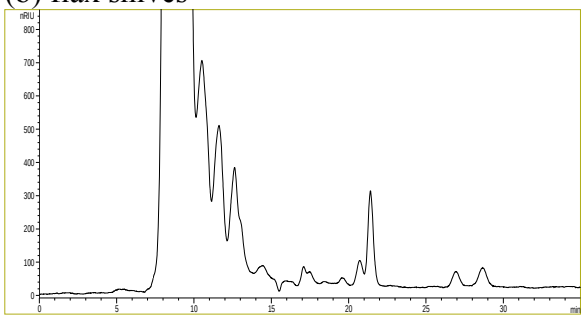


Fig. S1. Dissecting microscopic images (15× magnification) of the (**A**) oak wood, (**B**) flax shives, (**C**), ponderosa pine wood, (**D**) smooth sumac wood, (**E**) grape stem, and (**F**) triticale straw. Dissolution processes in [BMIM]Cl at 0 (upper row), 15 (middle row), and 60 seconds (lower row) microwave irradiation. Scale gradations are 1 mm.

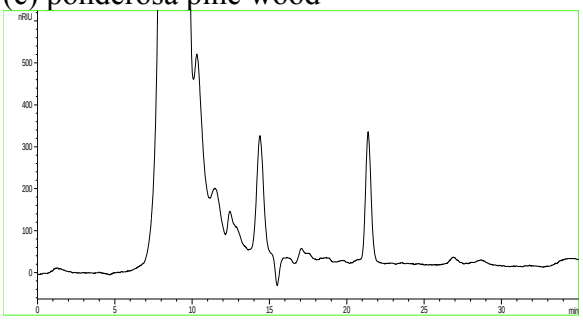
(a) oak wood



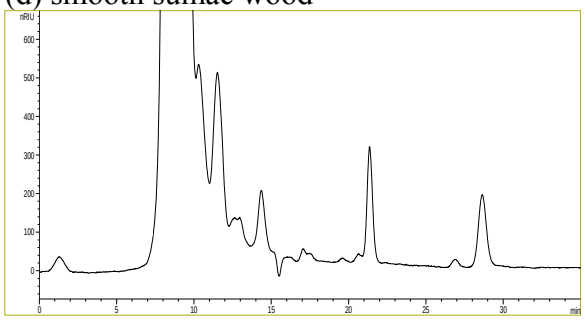
(b) flax shives



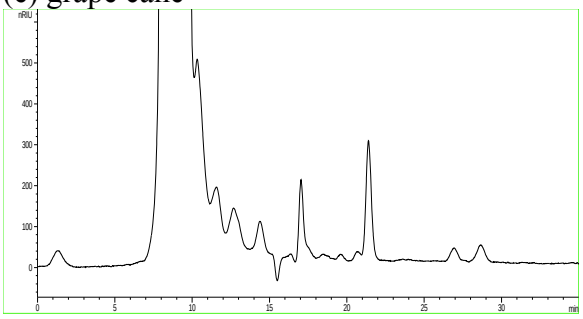
(c) ponderosa pine wood



(d) smooth sumac wood



(e) grape cane



(f) triticale straw

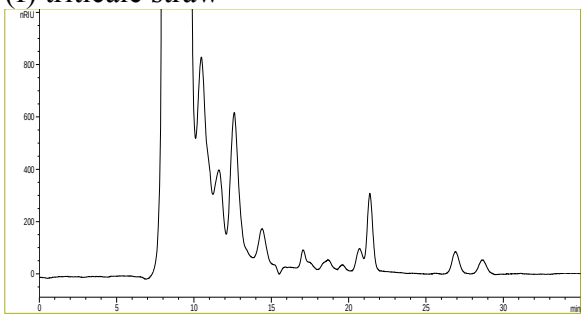


Fig. S2. Liquid chromatograms with refractive index detection of liquefied (a) oak wood, (b) flax shives, (c) ponderosa pine wood, (d) smooth sumac wood, (e) grape cane, and (f) triticale straw. Samples were 5 wt. % solutions in $[C_4MIM]Cl$ diluted 10:1 (v/v) in water and filtered through a 0.45 μm PVDF membrane prior to analysis.

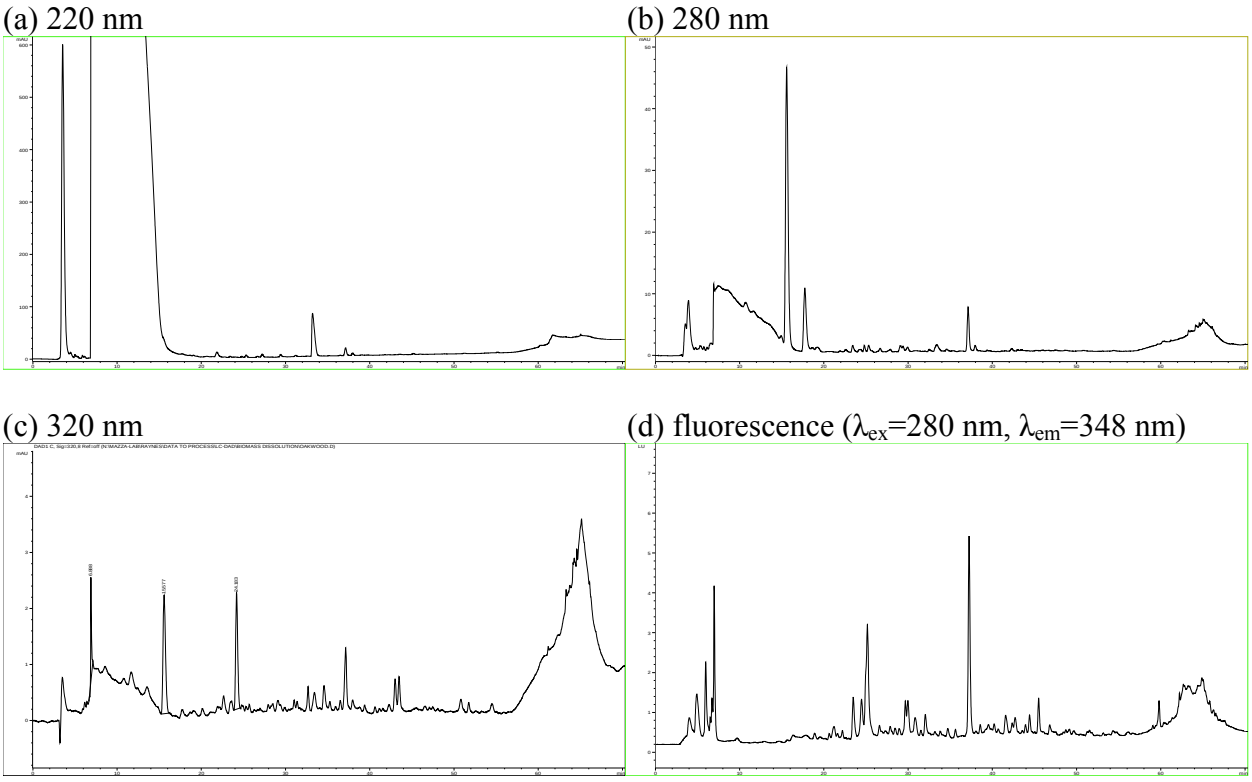


Fig. S3i. Liquid chromatography with diode array detection chromatograms of liquefied oak wood monitoring at (a) 220 nm, (b) 280 nm, (c) 320 nm, and (d) fluorescence detection (λ_{ex}=280 nm, λ_{em}=348 nm). Samples were 5 wt. % solutions in $[C_4MIM]Cl$ diluted 4:1 (v/v) in 4% sulfuric acid and filtered through a 0.45 μm PVDF membrane prior to analysis.

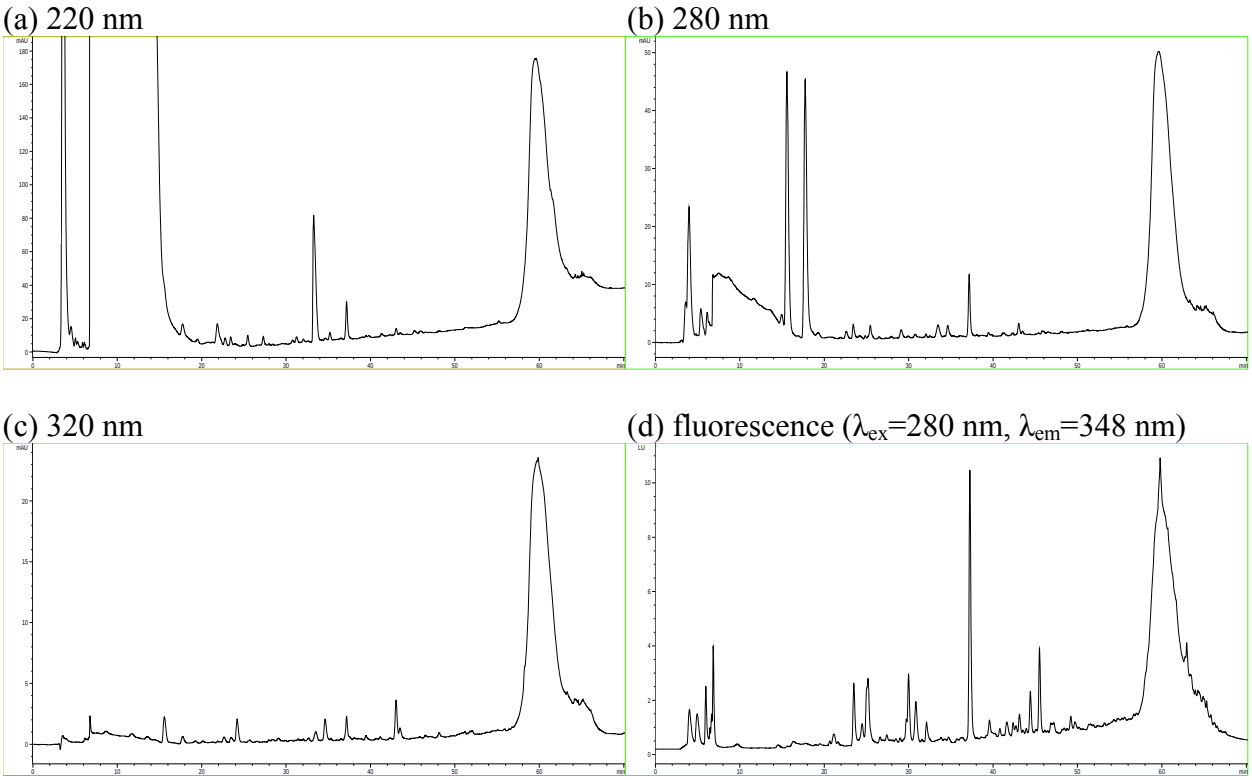


Fig. S3ii. Liquid chromatography with diode array detection chromatograms of liquefied flax shives monitoring at (a) 220 nm, (b) 280 nm, (c) 320 nm, and (d) fluorescence detection (λ_{ex}=280 nm, λ_{em}=348 nm). Samples were 5 wt. % solutions in [C_4MIM]Cl diluted 4:1 (v/v) in 4% sulfuric acid and filtered through a 0.45 μm PVDF membrane prior to analysis.

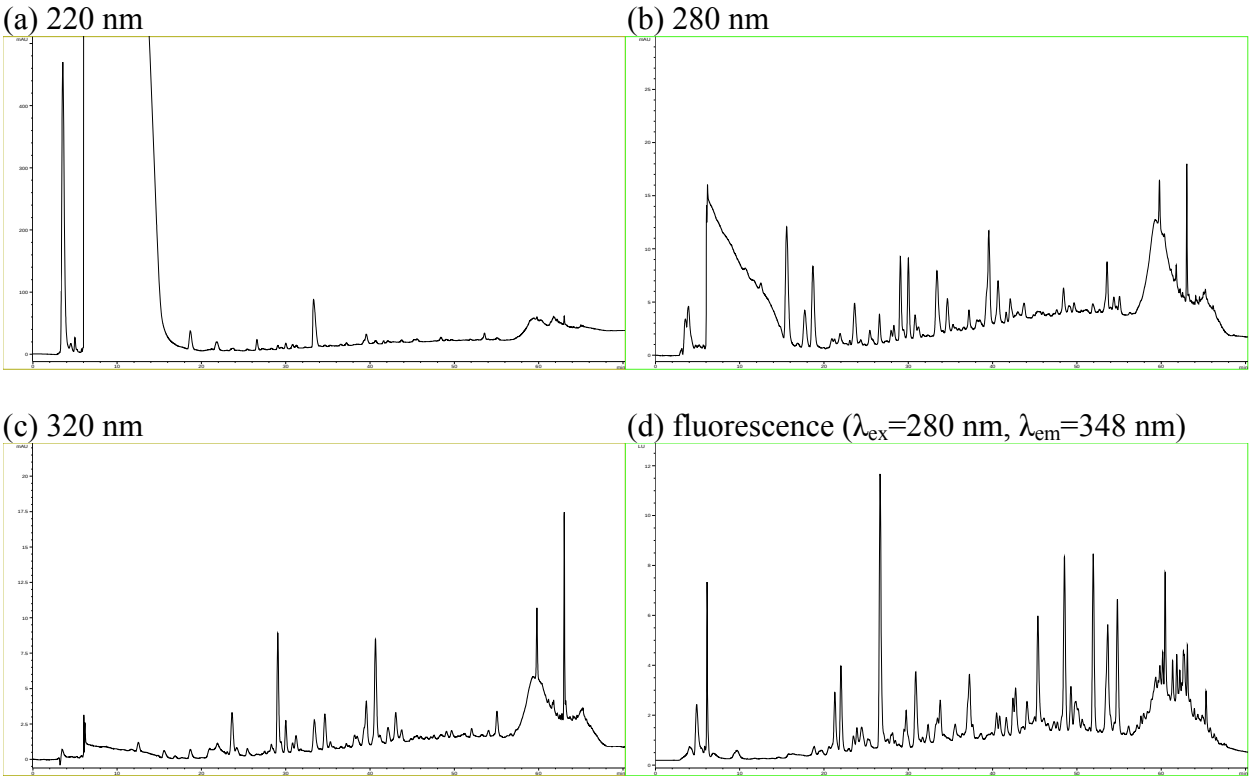


Fig. S3iii. Liquid chromatography with diode array detection chromatograms of liquefied ponderosa pine wood monitoring at (a) 220 nm, (b) 280 nm, (c) 320 nm, and (d) fluorescence detection (λ_{ex}=280 nm, λ_{em}=348 nm). Samples were 5 wt. % solutions in [C_4MIM]Cl diluted 4:1 (v/v) in 4% sulfuric acid and filtered through a 0.45 μm PVDF membrane prior to analysis.

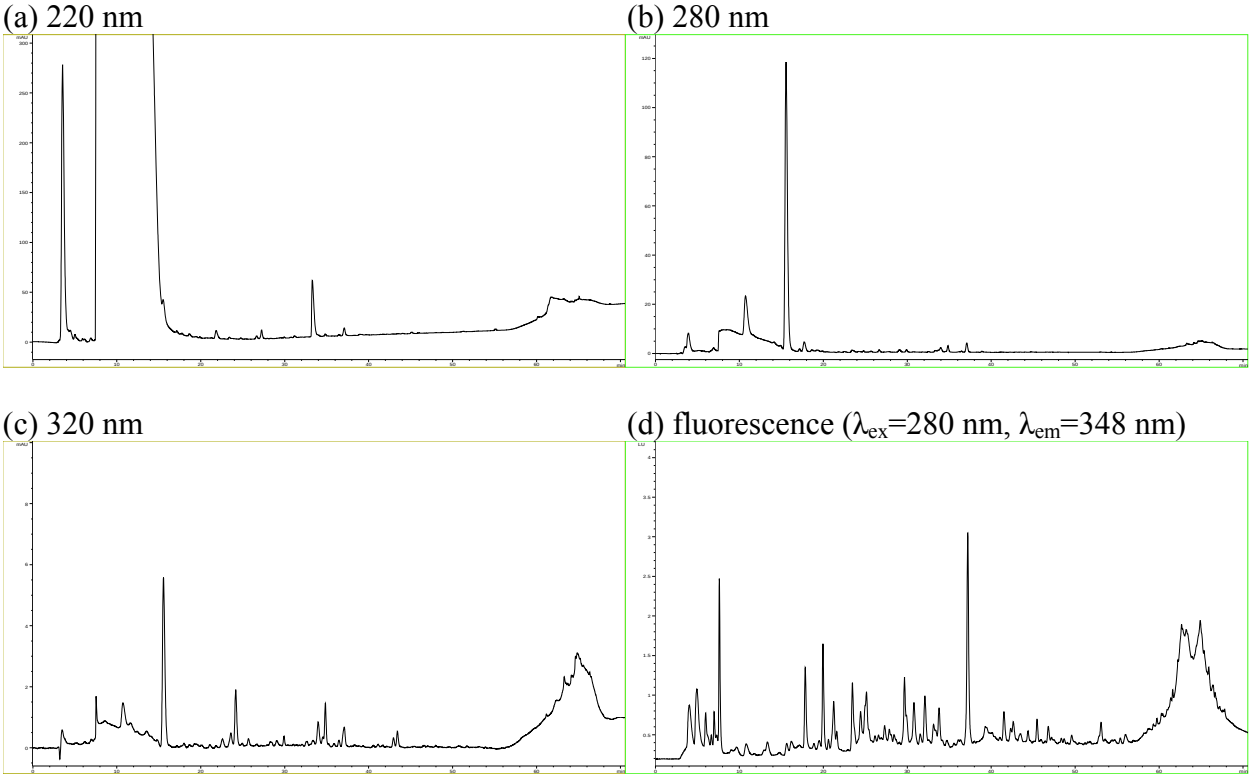


Fig. S3iv. Liquid chromatography with diode array detection chromatograms of liquefied smooth sumac wood monitoring at (a) 220 nm, (b) 280 nm, (c) 320 nm, and (d) fluorescence detection (λ_{ex}=280 nm, λ_{em}=348 nm). Samples were 5 wt. % solutions in [C_4MIM]Cl diluted 4:1 (v/v) in 4% sulfuric acid and filtered through a 0.45 μm PVDF membrane prior to analysis.

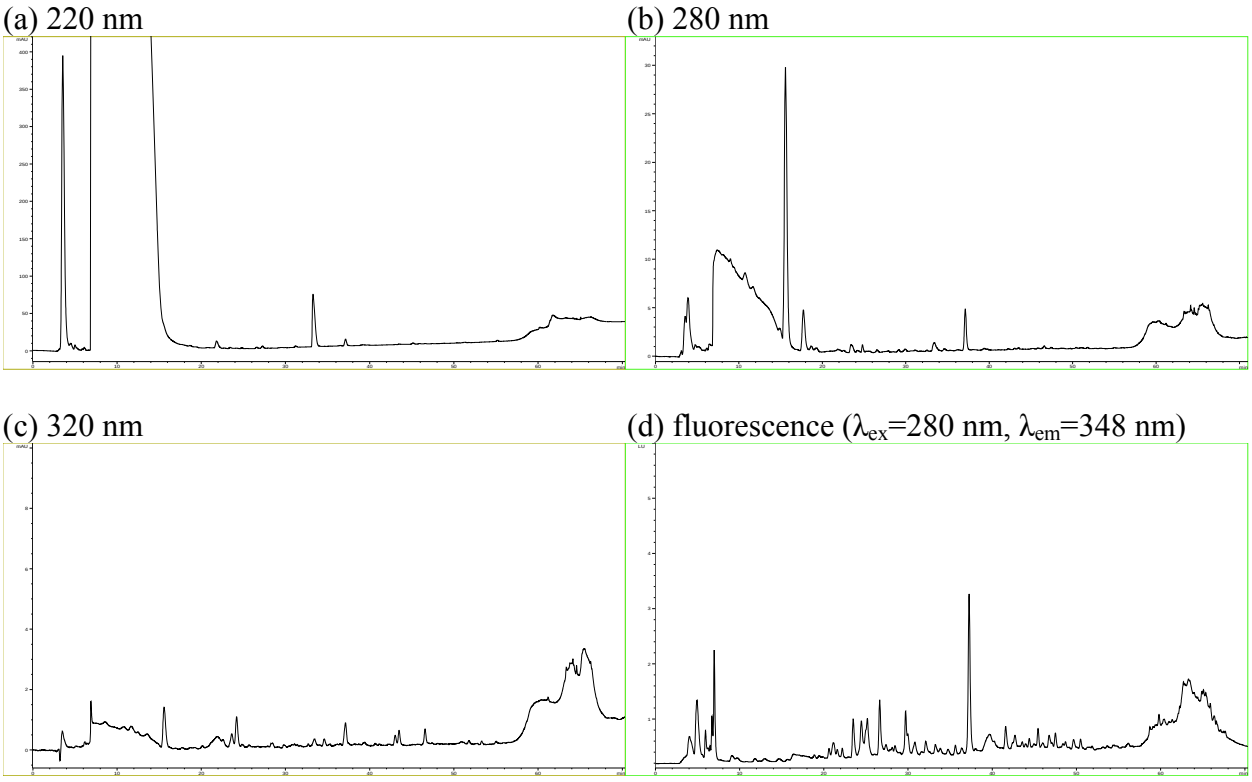


Fig. S3v. Liquid chromatography with diode array detection chromatograms of liquefied grape cane monitoring at (a) 220 nm, (b) 280 nm, (c) 320 nm, and (d) fluorescence detection (λ_{ex}=280 nm, λ_{em}=348 nm). Samples were 5 wt. % solutions in [C_4MIM]Cl diluted 4:1 (v/v) in 4% sulfuric acid and filtered through a 0.45 μm PVDF membrane prior to analysis.

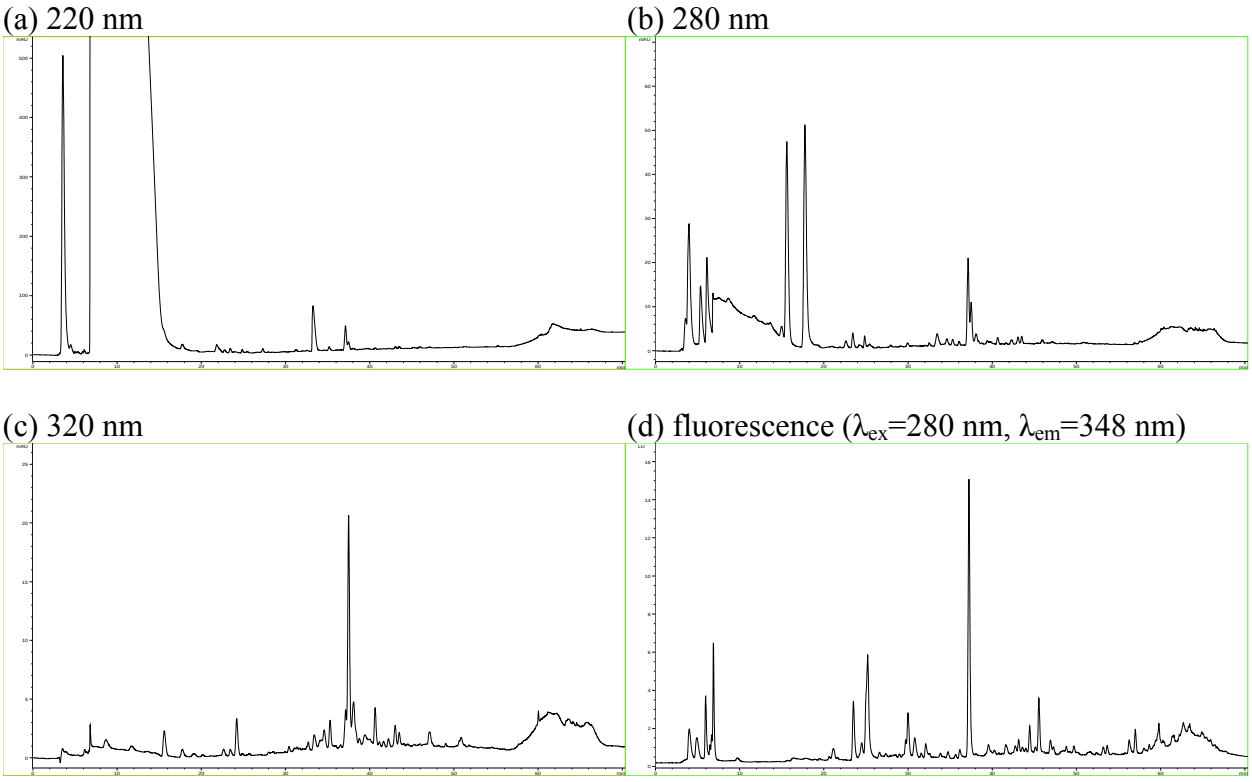
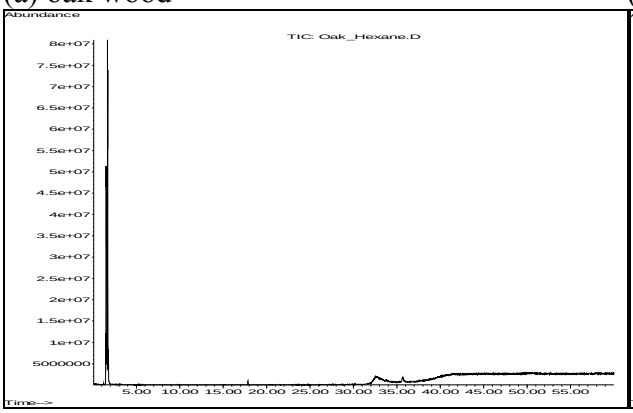
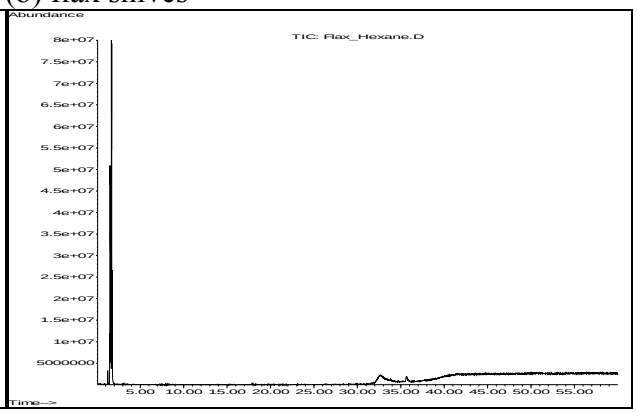


Fig. S3vi. Liquid chromatography with diode array detection chromatograms of liquefied triticale straw monitoring at (a) 220 nm, (b) 280 nm, (c) 320 nm, and (d) fluorescence detection (λ_{ex}=280 nm, λ_{em}=348 nm). Samples were 5 wt. % solutions in [C_4MIM]Cl diluted 4:1 (v/v) in 4% sulfuric acid and filtered through a 0.45 μm PVDF membrane prior to analysis.

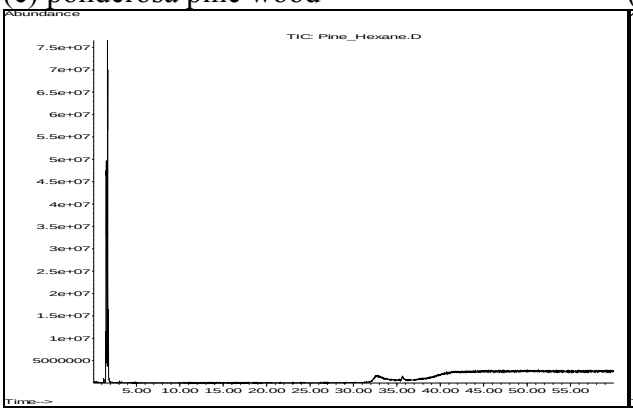
(a) oak wood



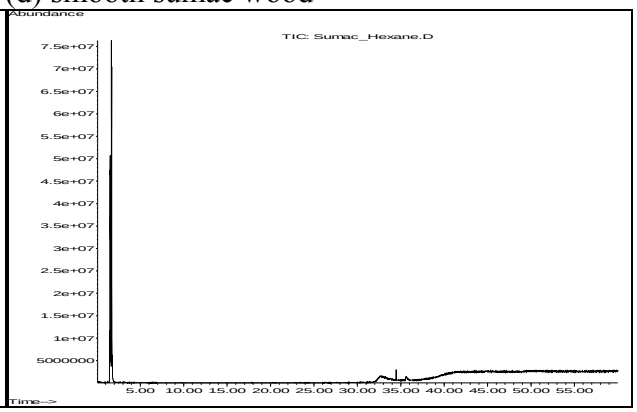
(b) flax shives



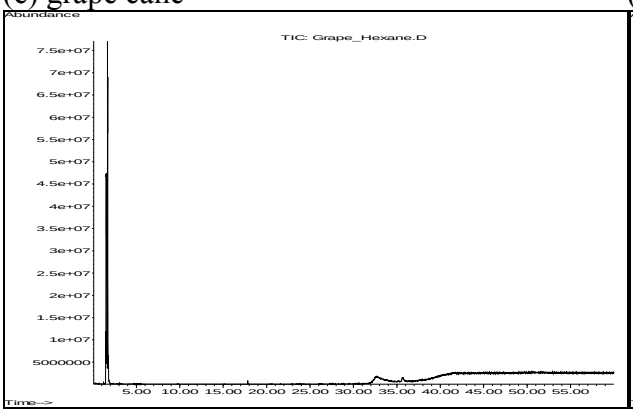
(c) ponderosa pine wood



(d) smooth sumac wood



(e) grape cane



(f) triticale straw

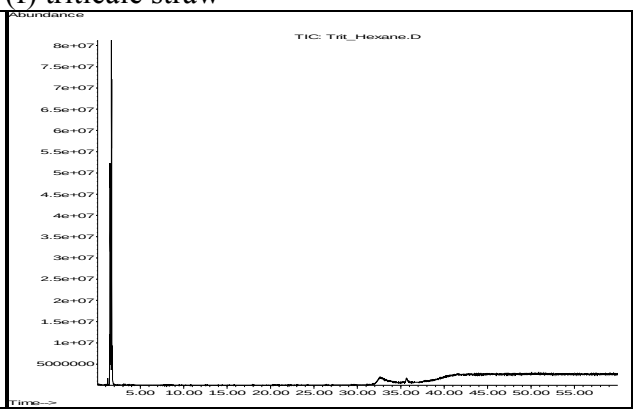


Fig. S4. Total ion GC-MS chromatograms (Σ*m/z* over the range from 50 to 60 in positive ion mode using 70 eV electron impact ionization) of liquefied (a) oak wood, (b) flax shives, (c) ponderosa pine wood, (d) smooth sumac wood, (e) grape cane, and (f) triticale straw. Samples were 5 wt. % solutions in $[C_4MIM]Cl$ extracted 1:1 (v/v) with hexane and filtered through a 0.45 μm PVDF membrane prior to analysis.

4. Supporting Information References

1. B. Hames, *Preparation of Samples for Compositional Analysis*, United States Department of Energy, Washington, DC, USA, 2005.

2. A. Sluiter, *Determination of Total Solids in Biomass*, United States Department of Energy, Washington, DC, USA, 2005.

3. A. Sluiter, *Determination of Extractives in Biomass*, United States Department of Energy, Washington, DC, USA, 2005.

4. A. Sluiter, *Determination of Structural Carbohydrates and Lignin in Biomass*, United States Department of Energy, Washington, DC, USA, 2006.

5. A. Sluiter, *Determination of Ash in Biomass*, United States Department of Energy, Washington, DC, USA, 2005.