Combined extraction/purification-catalytic microwave-assisted conversion of *Laurus nobilis* L. pruning waste polysaccharides into methyl levulinate

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SUPPORTING INFORMATION

METHODS

Carbohydrate content determination

Methanolysis was performed with 2M HCl in methanol on liquors and polysaccharides (1 mg of freeze-dried material) in triplicate at 100 ºC for 5h. Samples were neutralized with pyridine (approx. 200 µL), cooled and dried to room temperature and then hydrolyzed by 2M TFA hydrolysis at 120 ºC for 1h. Monosaccharides were separated and quantified by HPAEC-PAD on an ICS3000 system (Dionex, Sunnyvale, CA) using a Dionex CarboPac PA1 column at 30 ºC at a flow rate of 1 mL/min. Two different gradients were applied for the analysis of neutral sugars (fucose, arabinose, rhamnose, galactose, glucose, xylose and mannose) and uronic acids (galacturonic, glucuronic and 4-*O*-methyl-D-glucuronic acids). Acid hydrolysis was performed as follow. Briefly, 4 mg of freeze-dried sample (in triplicate) were hydrolyzed using 72% H_2SO_4 for 1h at room temperature, followed by 1M H_2SO_4 for 3h at 100 ºC. The hydrolyzed samples were filtered through a Chromacol 0.2 µm filter (Thermo Scientific, Carlsbad, CA, USA) and analyzed by HPAEC-PAD following the same method as for liquors and hemicelluloses.

HPLC analysis

Prior to the analysis, samples were subjected to a saponification treatment. Briefly, 10 mg of sample (freeze-dried) were mixed with 2M NaOH, flashed with N_2 and left to stand at 27 ºC overnight. Then, the saponified solution was adjusted to pH 3.0 using 12M HCl. The mixtures were centrifuged, and the supernatant was recovered to be analyzed using the ZORBAX StableBond C18 column (Agilent Technologies, USA) fitted to a separation module (Waters 2695, MA, USA) coupled to a photodiode array detector (Waters 2996, MA, USA) at 200-400 nm.

Molecular mass distribution

The molecular mass distribution of the polymers contained in liquors and the polysaccharide fractions was determined by size-exclusion chromatography (SEC) in a SECurity 1260, Polymer Standard Services (Mainz, Germany) coupled to a refractive index detector at 45 ºC. Prior to SEC injection, the samples were dissolved in the SEC eluent consisting of dimethyl sulfoxide (DMSO, HPLC grade, Scharlab, Sweden) with 0.5% w/w LiBr (ReagentPlus) at 60 ºC overnight. Analyses were performed in the system at a flow rate of 0.5 mL min^{-1} at 60 ºC using a column set consisting of a GRAM PreColumn, 30 and 10,000 analytical

columns (Polymer Standards Services, Germany). Standard calibration of the SEC separation was performed using pullulan standards ranging between 342 and 708,000 Da provided by Polymer Standards Services (Germany). The conversion of the elution volume into a size parameter was performed using the Mark-Houwink equation for pullulan using the Mark-Houwink parameters of K= 2.427 x 10^{-4} dL g^{-1} and a = 0.6804 (for pullulan in DMSO/LiBr). The DRI detector directly provides the SEC weight distribution as a function of the hydrodynamic radius. Processing of the MALLS data was performed using WinGPC Siftware (Polymer Standards Services, Germany) using the Zimm approximation and a dndc value of 0.0691 mL g^{-1}.

GC analysis during MLA obtention tests

The reaction mixture was filtered and analyzed by gas chromatography (GC) in an Agilent 7890A gas chromatograph using a flame ionization detector (FID) and a capillary column, HP-5 (Petrocol 2-4160: 300 ºC, 100 m x 250 µm x 0.5 µm). Calibration curves were obtained with an internal standard method using decane as standard. Standard solutions of MLA (0.0137-0.2348 M) and 0.150 M octane in decane were analyzed by GC to give a linear regression with R^2 >0.999. Gas chromatography-Mass spectroscopy (GC-MS) analysis was also performed using an Agilent 7820 A GC/5977B High Efficiency Source (HES) MSD (Madrid, Spain), in order to identify the obtained MLA in comparison with commercially available standard.

Parameters of the GC analysis were: starting T 150 ºC (hold 1'), ramp 25 ºC/min to 200 ºC (hold 1 min), heat 10 ºC/min to 250 ºC (hold 10 min), heat 25 ºC/min to 280 ºC (hold 20 min). FID 300 ºC, H_2 flow 30 mL/min, air flow 270 mL/min, makeup flow 25 mL/min. Inlet: 200 ºC, 28 psi.

Table S1. Liquors characterization at different auto-hydrolysis time and temperatures.

		Log R_0	pH	Density (g/L)	TDS (g/L)	Inorganic matter (g/L)	Organic matter (g/L)	Solid yield (%)	Polysaccharide yield (%)
160 ºC	**30'**	3.24	4.24 ± 0.01	986.9 ± 0.7	24.4 ± 0.1	1.7 ± 0.2	22.7 ± 0.2	24.9	2.56
	45'	3.42	4.13 ± 0.01	987.0 ± 3.5	27.9 ± 0.3	2.1 ± 0.01	25.8 ± 0.3	28.0	2.39
	60'	3.54	4.04 ± 0.02	986.5 ± 6.6	29.0 ± 0.6	2.3 ± 0.01	26.7 ± 0.2	29.8	2.42
	75'	3.64	4.09 ± 0.03	980.4 ± 5.9	29.0 ± 1.0	3.3 ± 0.7	25.7 ± 1.3	33.2	2.37
180 ºC	**30'**	3.83	3.93 ± 0.01	987.5 ± 4.9	30.8 ± 0.3	2.3 ± 0.6	28.5 ± 0.7	39.1	0.97
	45'	4.01	3.85 ± 0.01	984.4 ± 7.4	27.9 ± 0.1	3.6 ± 0.001	24.4 ± 0.7	40.3	0.41
	60'	4.13	3.79 ± 0.06	981.2 ± 6.1	25.6 ± 0.5	2.0 ± 1.1	23.6 ± 1.0	36.6	0.21
	75'	4.23	3.81 ± 0.03	978.9 ± 8.2	22.7 ± 0.2	1.6 ± 0.1	21.1 ± 0.1	41.0	0.01
190 ºC	**30'**	4.13	3.06 ± 0.02	975.6 ± 7.2	24.6 ± 0.1	4.0 ± 0.5	20.5 ± 0.6	41.8	0.27
	45'	4.30	3.69 ± 0.04	981.7 ± 1.5	22.6 ± 0.4	2.8 ± 0.4	20.0 ± 0.4	40.7	0.17
	60'	4.43	3.64 ± 0.03	978.4 ± 5.5	19.1 ± 0.1	2.1 ± 0.005	17.0 ± 0.0	42.1	0.08
	75'	4.52	3.63 ± 0.03	979.2 ± 5.6	17.0 ± 0.1	2.0 ± 0.002	15.0 ± 0.0	37.7	0.06
200 ºC	**30'**	4.42	3.66 ± 0.00	976.9 ± 3.6	19.2 ± 0.1	1.6 ± 0.7	18.1 ± 1.1	42.1	0.09
	45'	4.60	3.57 ± 0.00	977.2 ± 1.6	17.1 ± 0.2	3.2 ± 0.4	14.0 ± 0.4	42.1	0.05
	60'	4.72	3.77 ± 0.01	978.6 ± 3.2	16.4 ± 0.1	5.3 ± 0.04	11.2 ± 0.5	42.3	0.02
	75'	4.82	3.67 ± 0.03	976.2 ± 1.2	14.2 ± 0.1	1.7 ± 0.2	12.5 ± 0.2	43.5	0.01

Table S2. Carbohydrate composition, molar mass distribution, protein content, and phenolic acids determination in BTPW and the obtained liquors.

														Phenolic acids		
		Total Carbohydrates (mg/g)	Fucose (mg/g)	Arabinose (mg/g)	Rhamnose (mg/g)	Galactose (mg/g)	Glucose (mg/g)	Xylose (mg/g)	Mannose (mg/g)	Uronics (mg/g)[a]	Mn (KDa)	Mw (KDa)	Protein content (mg/g)	Coumaric acid (mg/g)	Ferulic acid (mg/g)	Caffeic acid (mg/g)
BTPW		451.70	2.04	37.06	-	29.77	236.68	107.09	7.37	31.69	NA	NA	-	3.66	1.96	ND
160 ºC	30’	309.53	0.88	74.75	23.91	23.58	124.55	42.33	11.22	8.30	1.15	4.92	7.20	0.52	0.82	ND
	45’	328.86	0.88	49.97	26.36	21.77	133.66	72.63	11.04	12.54	1.23	4.05	6.22	1.89	ND	ND
	60’	269.02	1.05	38.52	17.15	18.23	98.36	76.27	9.73	9.71	1.46	3.73	5.84	0.49	ND	ND
	75’	287.54	0.75	29.91	21.85	16.64	107.13	91.60	11.34	8.31	1.44	3.72	5.51	1.12	0.97	ND
180 ºC	30’	244.10	0.55	19.99	16.43	12.78	76.22	99.44	11.54	7.15	1.21	2.58	4.44	0.91	0.80	ND
	45’	188.38	0.38	10.37	8.44	10.66	66.92	76.83	10.17	4.61	1.17	1.87	8.54	1.11	0.84	ND
	60’	122.05	0.73	5.20	4.74	8.11	45.19	46.02	6.96	5.11	1.20	1.79	4.36	ND	1.06	ND
	75’	158.49	0.38	7.12	4.32	10.91	50.33	69.82	11.87	3.75	1.06	1.63	6.75	ND	ND	0.34
190 ºC	30’	160.19	0.27	9.05	3.73	8.99	55.95	66.50	11.32	4.38	1.16	1.79	5.13	ND	1.04	0.45
	45’	110.47	0.18	3.80	2.20	6.89	42.51	42.65	9.79	2.45	0.96	1.41	5.40	ND	1.34	1.06
	60’	83.66	0.19	1.98	2.60	5.87	41.37	21.34	6.75	3.56	0.92	1.27	6.59	ND	2.63	2.08
	75’	56.13	0.20	1.12	2.64	4.32	30.29	10.01	5.09	2.46	1.04	1.47	5.38	ND	2.43	2.61
200 ºC	30’	75.94	0.14	1.49	1.69	4.18	31.55	17.83	6.58	12.48	1.09	1.49	6.08	ND	2.24	1.77
	45’	47.44	0.18	0.30	1.92	2.45	22.07	5.05	3.87	11.59	1.18	1.62	6.72	ND	1.70	2.82
	60’	26.05	0.73	0.59	0.44	1.06	8.44	1.37	1.12	12.30	1.12	1.54	5.10	ND	2.73	1.57
	75’	26.84	0.40	0.27	0.19	0.38	4.33	0.40	0.22	20.65	1.12	1.50	5.03	ND	3.49	2.84

[a]Sum of GalA, GlcA and OMeGlcA

Table S3. Carbohydrate composition, molar mass distribution, and phenolic acids determination in polysaccharides and the remaining residues after autohydrolysis.

															Phenolic acids (mg/g)			
Polysaccharides		Total Carbohydrates (mg/g)	α-glucans (mg/g)	β-glucans (mg/g)	Fucose (mg/g)	Arabinose (mg/g)	Rhamnose (mg/g)	Galactose (mg/g)	Glucose (mg/g)	Xylose (mg/g)	Mannose (mg/g)	Uronics (mg/g)[a]	Mn (KDa)	Mw (KDa)	Coumaric acid	Ferulic acid	Caffeic acid	Sinapic acid
	30 min	531.64	273.34	0.36	0.35	21.23	13.29	61.02	273.70	141.58	13.18	7.30	8.93	71.57	0.10	ND	ND	ND
160 ºC	45 min	429.98	180.91	0.39	0.13	7.30	7.60	45.44	181.30	172.41	9.5	6.46	6.02	183.4	0.60	0.12	ND	ND
	60 min	272.25	122.19	0.37	0.10	3.23	3.32	26.93	122.56	108.05	2.27	6.30	6.97	128.3	0.03	0.01	ND	ND
	75 min	233.95	104.56	0.41	0.09	1.59	1.54	22.55	104.95	92.62	2.15	8.47	6.10	104.00	0.03	ND	ND	ND
	30 min	116.42	70.75	0.48	0.07	0.65	1.06	12.74	71.23	13.51	0.42	16.75	4.17	8.20	0.79	0.13	ND	ND
180 ºC	45 min	88.25	55.94	0.29	0.17	0.36	0.74	9.19	56.23	6.72	1.07	13.76	4.46	6.64	ND	ND	ND	ND
	60 min	54.81	24.44	0.09	0.11	0.35	0.29	11.65	24.53	5.64	0.61	11.63	4.77	5.75	0.18	0.14	0.17	ND
	75 min	16.93	3.52	0.06	0.03	0.14	0.17	0.60	3.98	4.27	1.05	6.68	2.16	3.13	1.24	0.45	0.16	ND
	30 min	33.56	12.99	0.12	0.06	0.35	0.13	1.97	13.11	5.27	1,.06	11.62	4.59	5.40	0.15	0.18	0.08	ND
190 ºC	45 min	8.59	2.88	0.07	0.05	0.11	0.13	0.49	2.95	2.37	0.65	1.84	3.97	4.30	ND	0.20	0.15	ND
	60 min	17.73	1.17	0.06	0.05	0.08	0.07	0.32	1.23	0.52	0.58	13.89	0.58	0.74	ND	0.21	0.22	ND
	75 min	11.06	0.48	0.10	0.07	0.06	0.12	0.17	0.58	0.52	0.53	9.02	0.57	0.62	ND	0.20	0.28	ND
	30 min	12.29	0.75	0.04	0.01	0.09	0.09	0.22	0.79	0.68	0.38	10.03	1.05	1.36	0.10	0.30	0.30	ND
200 ºC	45 min	10.57	0.29	0.21	0.93	0.08	0.22	0.07	0.50	0.16	0.45	8.15	0.74	0.87	ND	0.34	0.48	ND
	60 min	11.87	0.27	0.16	0.73	0.07	0.13	0.13	0.43	0.18	0.27	9.06	0.25	0.31	ND	0.23	0.23	ND
	75 min	4.93	NM	NM	2.09	0.13	0.63	0.33	0.55	0.49	0.70	ND	0.10	0.11	ND	ND	ND	ND
Residues																		
	30 min	43.46	NM	NM	0.07	0.85	0.46	0.90	23.37	17.16	0.59	0.07	NA	NA	3.98	3.06	ND	2.16
	45 min	26.86	NM	NM	0.03	0.33	0.25	0.71	15.62	9.53	0.33	0.05	NA	NA	7.76	2.66	ND	3.17
160 ºC	60 min	15.15	NM	NM	0.02	0.27	0.20	0.57	9.35	4.45	0.13	0.16	NA	NA	7.80	3.29	ND	3.75
	75 min	14.76	NM	NM	0.01	0.13	0.09	0.37	9.71	4.04	0.11	0.29	NA	NA	6.17	3.86	ND	3.12
	30 min	9.79	NM	NM	0.01	0.09	0.06	0.24	6.93	2.38	0.05	0.03	NA	NA	5.65	3.08	ND	3.41
180 ºC	45 min	8.77	NM	NM	0.01	0.02	0.01	0.12	6.95	1.57	0.06	0.03	NA	NA	8.13	2.90	ND	4.35
	60 min	7.35	NM	NM	0.01	0.01	0.01	0.04	5.91	1.07	0.02	0.27	NA	NA	5.37	2.13	ND	2.22
	75 min	4.58	NM	NM	0.01	ND	ND	0.04	3.51	0.75	0.01	0.24	NA	NA	5.50	3.35	ND	2.96
	30 min	2.91	NM	NM	0.01	0.01	0.02	0.06	2.26	0.50	0.01	0.03	NA	NA	3.95	2.78	ND	2.25
190 ºC	45 min	4.02	NM	NM	0.01	ND	0.01	0.02	3.43	0.44	0.01	0.09	NA	NA	2.97	2.63	ND	2.66
	60 min	3.49	NM	NM	0.01	ND	0.01	0.01	2.10	0.21	0.01	1.14	NA	NA	1.40	2.42	ND	4.21
	75 min	4.57	NM	NM	0.01	ND	0.01	0.01	3.11	0.21	ND	1.21	NA	NA	2.31	1.91	ND	2.43
	30 min	3.46	NM	NM	0.01	ND	ND	0.02	1.48	0.12	ND	1.82	NA	NA	4.04	3.34	ND	2.75
200 ºC	45 min	6.91	NM	NM	0.01	ND	0.01	0.01	2.34	0.11	ND	4.43	NA	NA	1.85	2.79	ND	1.51
	60 min	1.66	NM	NM	0.01	ND	0.01	ND	1.50	0.07	0.01	0.07	NA	NA	3.55	1.86	ND	5.37
	75 min	1.82	NM	NM	0.01	ND	ND	ND	1.64	0.07	ND	0.09	NA	NA	3.11	2.08	ND	2.08

[a]Sum of GalA, GlcA and OMeGlcA

Table S4. MW-assisted synthesis of MLA.

Entry	MLA (mmol)	MLA (mg)	MLA (g)/feedstock (kg)	MLA (mg)/carbohydrates (g)
BTPW	0.042	5.366 ± 0.220	75.570 ± 3.100	34.135 ± 1.401
Liquor	0.047	6.092 ± 0.298	85.799 ± 4.200	28.216 ± 1.381
Polysaccharides	0.214	27.803 ± 0.305	391.597 ± 4.300	186.122 ± 2.043
Residue	0.022	2.839 ± 0.348	39.989 ± 4.900	1.074 ± 0.132

Figure S1. MW parameters (p, T and power) for the reaction carried out at fixed power.

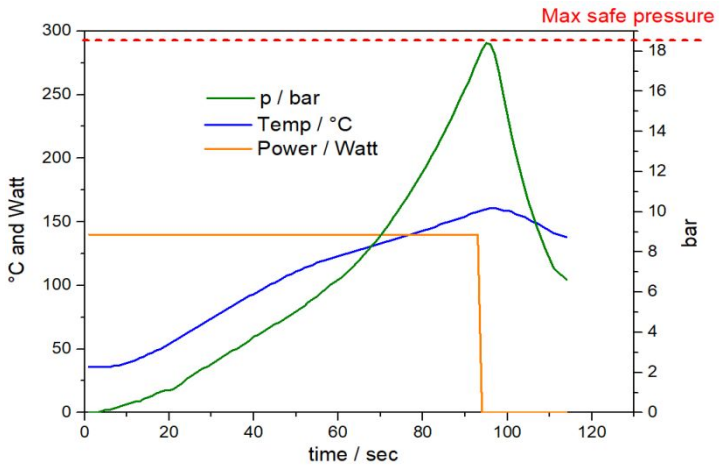

Figure S2. MW parameters (p, T and power) for the reaction carried out at fixed temperature.