Supporting Information

Delignification of olive tree pruning biomass using a ternary eutectic solvent for enhanced saccharification and isolation of a unique lignin fraction

*Irene Gómez-Cruz[a,b], Nalin Seixas[a], Jalel Labidi[c], Eulogio Castro[b], Armando J.D. Silvestre[a], André M. da Costa Lopes[a,d]**

[a]CICECO, Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.

[b]Department of Chemical, Environmental and Materials Engineering, Universidad de Jaén, Campus Las Lagunillas, 23071 Jaén, Spain.

[c]Department of Chemical and Environmental Engineering, University of the Basque Country UPV/EHU, Plaza Europa 1, 20018 Donostia-San Sebastian, Gipuzkoa, Spain.

[d]CECOLAB, Collaborative Laboratory Towards Circular Economy, R. Nossa Senhora da Conceição, 3405-155 Oliveira do Hospital, Portugal.

*Corresponding author: andremcl@ua.pt

Number of pages: 17

Number of figures: 6

Number of tables: 5

Table of contents

1. Materials and methods

1.1. Reagents

Ethylene glycol (EG) (≥99 % w/w), pyridine (99.8 % w/w), cyclohexanol (for synthesis), chromium (III) acetylacetonate (99.9 % w/w) and 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP, 95 % w/w) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Deuterium oxide (D2O; 99.9 % w/w), dimethylsulfoxide-D6 (DMSO-D6; 99.8 % w/w) and chloroform-D (99.8 % w/w) was ordered from Eurisotop (St. Aubin, Cedex, France). Cholinium chloride (ChCl) (99 % w/w) and absolute ethanol was obtained from Thermo Scientific Chemicals (Waltham, MA, USA) and Honeywell (Morristown, NJ, USA), respectively. p-toluenesulfonic acid (pTSA) (≥98 % w/w) and Simplicity® system for obtaining ultrapure water were purchased by VWR International (Radnor, PA, US).

1.2.Gel permeation chromatography (GPC)

Gel Permeation Chromatography (GPC) was used for the determination of the molecular weight and polydispersity index of lignin samples. A Jasco LC-Net II/ADC device equipped with a RI-2031 Plus Intelligent refractive index detector and two PolarGel-M columns (300 mm x 7.5 mm) placed in series PolarGel-M guard (50 mm 7.5 mm) was used. Approximately 20 µl of 50 ppm lignin solution dissolved in dimethylformamide with 0.1% lithium bromide was injected for each measurement. The same solvent was also used as the mobile phase of the column. The column was eluted with the mobile phase at 40 °C and a flow rate of 0.7 mL·min^{-1}. With molecular weights ranging from 266 to 70,000 g·mol^{-1}, polystyrene was used as the standard for the calibration curve.

1.3.HPLC analysis

The liquid samples were analyzed by HPLC (Elite LaChrom VWR HITCHI, Tokyo, Japan) to determine the content of sugars and/or sugar degradation products (furfural and HMF). The samples (10 µL) were injected into the HPLC system consisting of a pump L-2130 (Hitachi, Chiyoda, Japan), an autosampler L-2200 (Hitachi, Chiyoda, Japan) and two detectors, a photodiode array detector (PAD) L-2455 (Hitachi, Chiyoda, Japan) and a refractive index (RI) detector L-2490 (Hitachi, Chiyoda, Japan). The compounds were separated on a Rezex ROA-Organic acid H^+ 8% ion exchange column (300 x 7.8 mm; particle size 8 µm), supplied by Phenomenex (Torrance, CA, USA) and maintained at 65 °C (Gecko 2000 oven, CIL Cluzeau, Sainte-Foy-la-Grande, France). The eluent used was a 0.005 N solution of sulfuric acid at a flow rate of 0.5 $mL \cdot min^{-1}$. Calibration curves of glucose, xylose, furfural and HMF were used to determine the concentration of the analytes in the obtained liquid samples.

2. Figures

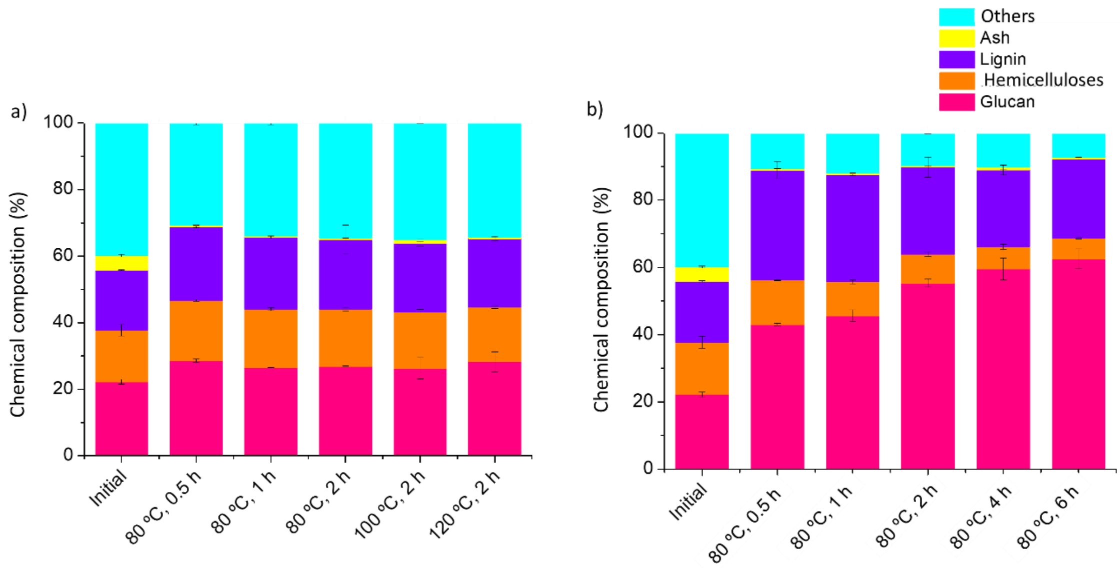

Figure S1. Chemical composition of main fractions after OTP treatment with a) binary (ChCl:EG) and b) ternary (ChCl:pTSA:EG) eutectic solvents. The data is expressed as mean ± standard deviation (n = 3).

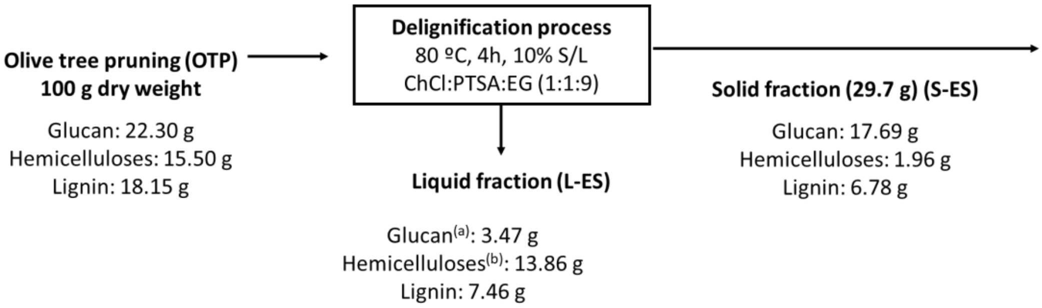

Figure S2. Mass balance of OTP delignification process with ChCl:pTSA:EG (1:1:9) at 80 °C and 4 h. a) glucose form, b) pentoses form.

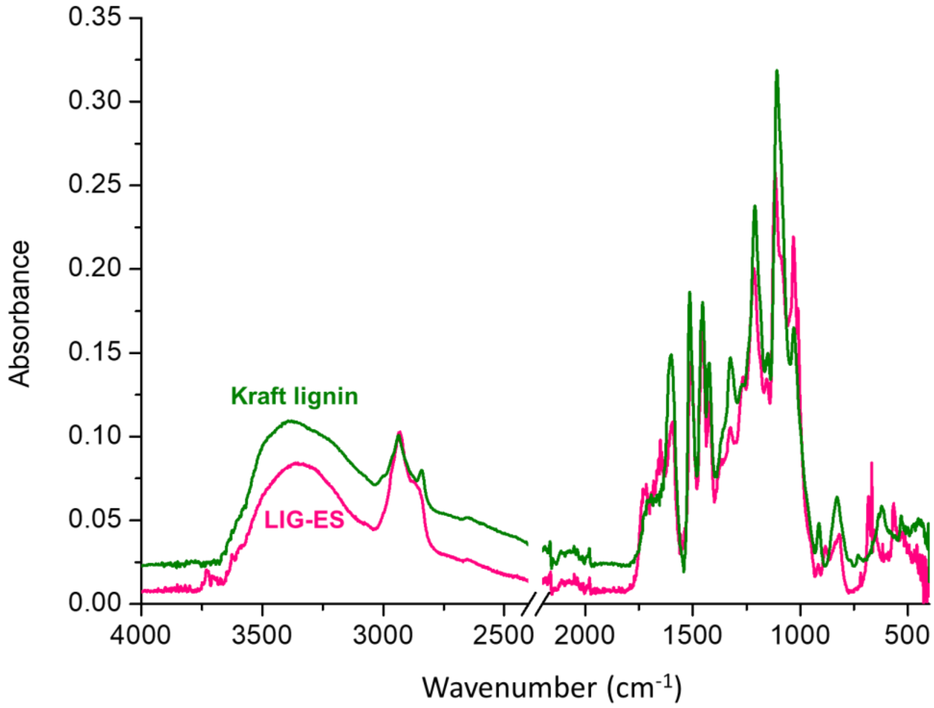

Figure S3. FTIR spectra of LIG-ES after extraction with ChCl:pTSA:EG eutectic solvent (80 ºC, 4h) and Kraft lignin.

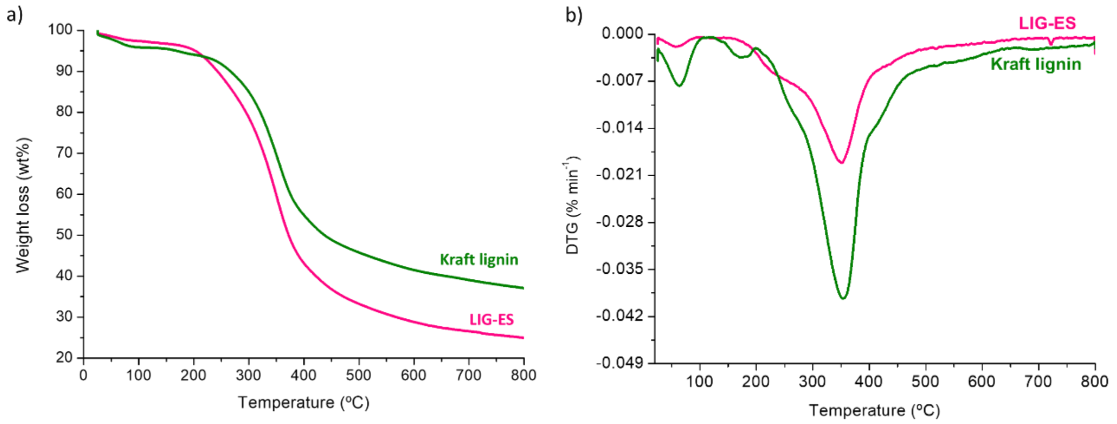

Figure S4. a) TGA and b) DTGA curves of LIG-ES after OTP fractionation with ChCl:pTSA:EG (80 ºC, 4h) and Kraft lignin.

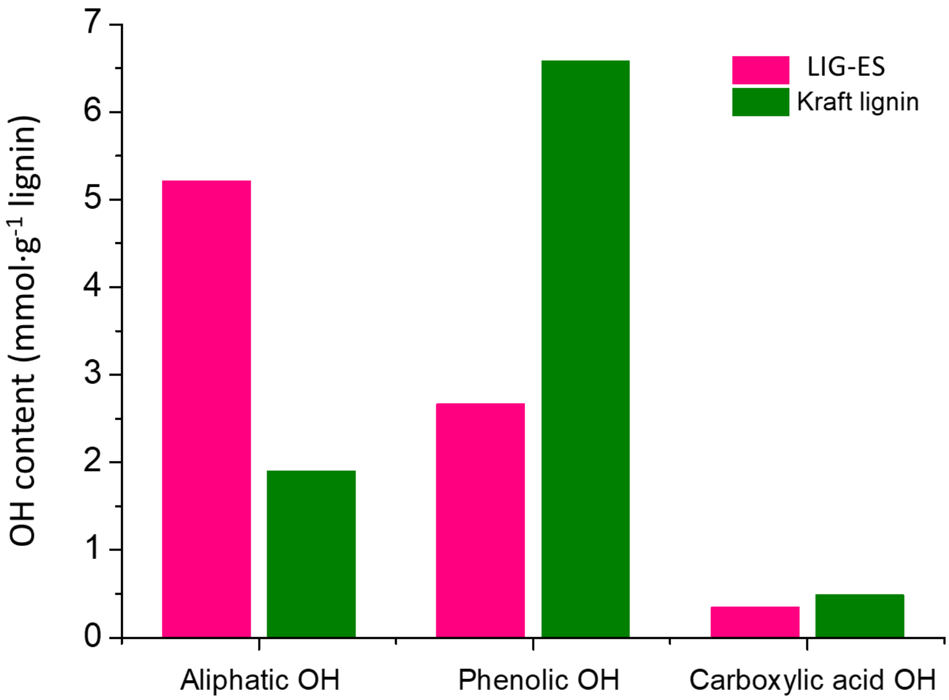

Figure S5. Contents of different hydroxyl and carboxylic groups in LIG-ES after OTP fractionation with ChCl:pTSA:EG (80 ºC, 4 h) and Kraft lignin determined by ^{31}P-NMR.

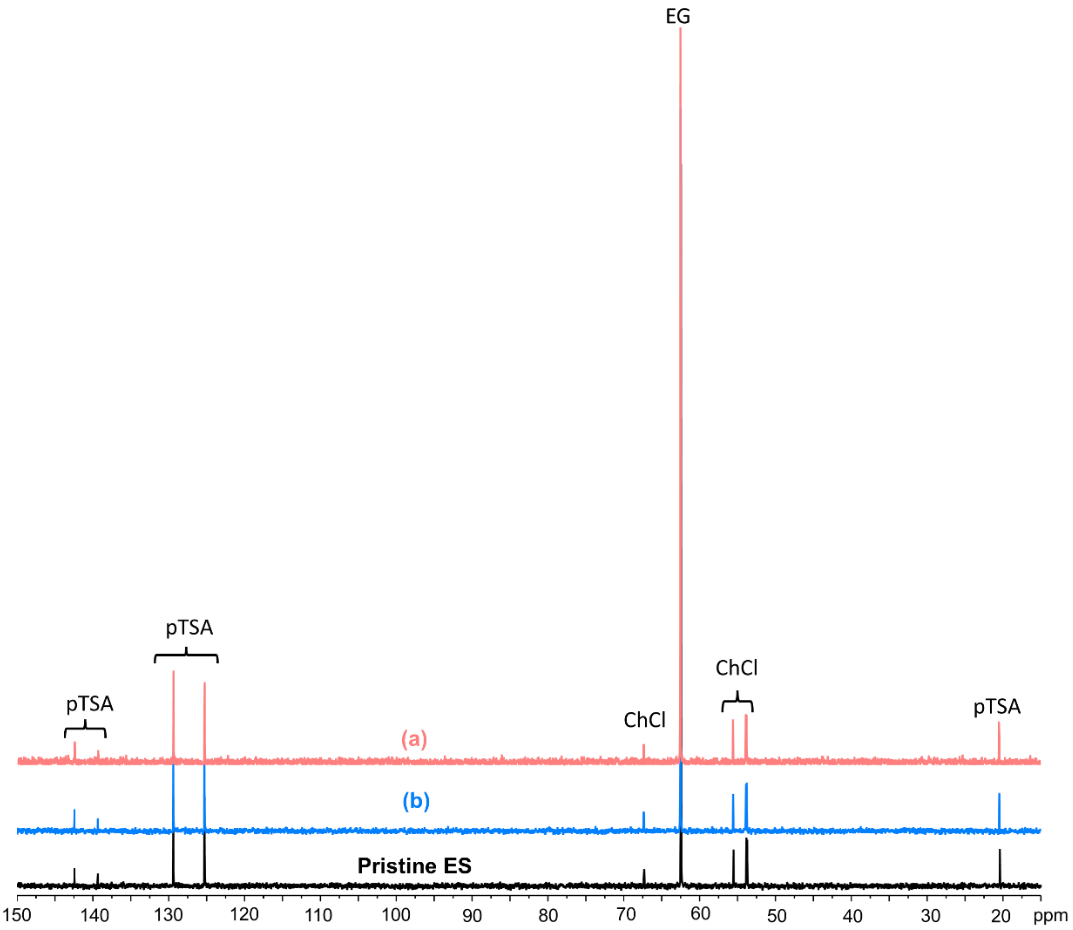

Figure S6. ^{13}C NMR spectra for pristine ES (after thermal heating at 80 ºC and 4 h), as well as recovered ES obtained after OTP fractionation (a) and adsorption (b).

3. Tables

Table S1. Liquid fraction composition after OTP fractionation with ChCl:EG and ChCl:pTSA:EG.

	80 ºC, 0.5 h	**80 ºC, 1 h**	**80 ºC, 2 h**	**100 ºC, 2 h**	**120 ºC, 2 h**
			ChCl:EG		
Glucan	0.89 ± 0.01	0.94 ± 0.01	0.93 ± 0.00	1.00 ± 0.03	0.94 ± 0.00
Hemicelluloses	0.30 ± 0.00	0.33 ± 0.02	0.32 ± 0.00	0.41 ± 0.02	0.63 ± 0.00
Xylan	0.26 ± 0.02	0.27 ± 0.02	0.28 ± 0.00	0.34 ± 0.02	0.51 ± 0.01
Arabinan	0.04 ± 0.00	0.06 ± 0.01	0.04 ± 0.00	0.07 ± 0.00	0.12 ± 0.00
	80 ºC, 0.5 h	**80 ºC, 1 h**	**80 ºC, 2 h**	**80 ºC, 4 h**	**80 ºC, 6 h**
			ChCl:pTSA:EG		
Glucan	2.74 ± 0.10	2.93 ± 0.17	3.30 ± 0.10	3.47 ± 0.05	3.78 ± 0.07
Hemicelluloses	9.37 ± 0.70	10.73 ± 0.50	12.87 ± 0.43	13.86 ± 0.42	15.09 ± 0.25
Xylan	5.88 ± 0.50	6.90 ± 0.34	8.54 ± 0.25	9.28 ± 0.32	10.12 ± 0.14
Arabinan	3.49 ± 0.20	3.83 ± 0.16	4.33 ± 0.18	4.58 ± 0.10	4.97 ± 0.11

The data is expressed as mean ± standard deviation (n = 3) as g of sugar·100 g^{-1} of OTP; ES: eutectic solvents.

Table S2. Delignification yield (%) and LIG-ES (mg lignin·g^{-1} biomass) mediated by binary (ChCl:EG).

Time (h)	**Temperature (°C)**	**Delignification yield (%)**	**Isolated lignin (mg lignin·g^{-1} biomass)**
0.5	80	3.4 ± 1.4	9.9 ± 0.4
1	80	5.2 ± 1.5	9.9 ± 0.8
2	80	5.4 ± 2.2	9.1 ± 0.4
2	100	8.6 ± 1.6	12.5 ± 1.0
2	120	10.7 ± 1.4	16.4 ± 0.7

Table S3. FTIR vibrational bands and corresponding assignments for LIG-ES after extraction with ChCl:pTSA:EG (80 ºC, 4h) [16, 17, 18, 19, 23].

Vibrational band (cm^{-1})	**Assignments**
3350	Stretching vibration of hydroxyl group and hydrogen bonding
2920-2850	C–H stretching vibrations in methyl and methylene groups
1600	Aromatic skeletal vibration
1515-1425	Typical aromatic skeletal vibrations
1460	C–H bending vibrations in methyl and methylene groups
1327	Syringyl unit breathing with C=O stretching and condensed guaiacyl rings
1270	Guaiacyl ring breathing with C–O stretching
1212	C-C plus C-O plus C=O stretch; Guaiacyl condensed > Guaiacyl etherified)
1158	C–O stretch in ester group
912-1031	Guaiacyl ring C–H in plane and out of plane bending
833-1118	Syringyl ring C–H in-plane and out of plane deformations

Table S4. Assignments of the lignin ^{13}C-^{1}H correlation signals in the 2D HSQC NMR of LIG-ES after extraction ChCl:pTSA:EG (80 ºC, 4h).

Labels	C	H	Assignment
OA	14.96	0.88	C-H in CH_3 group of oleic acid
OA	22.62	1.27	C-H in CH_2 group near CH_3 of oleic acid
OA	27.07	2.00	C-H in CH_2 near double bond in oleic acid
OA	29.10	1.20	C-H in remaining CH_2 of oleic acid
OA	29.20	1.27	C-H in remaining CH_2 of oleic acid
OA	31.89	1.25	C-H in CH_2 group near CH_2 (22.62/1.27ppm) of oleic acid
OA	33.91	2.30	C-H in CH_2 group near carboxylate of oleic acid
DMSO	40.11	2.52	Dimethysulfoxide D6 (DMSO)
C_β (S)	52.10	3.62	C_β−H_β in phenylcoumarans (C) linked to S-units
B_β	53.71	3.12	C_β−H_β in resinol substructures (B)
C_β (G)	53.78	3.48	C_β−H_β in phenylcoumarans (C) linked to G-units
OCH_3	56.26	3.74	C−H in methoxyls
A_γ	59.41	3.57	C_γ−H_γ in β-O-4′ alkyl-aryl ethers (A)
I_γ	60.43	4.04	C_γ−H_γ in cynnamyl alcohol end-groups (I)
ChCl	62.72	3.41	Coline chloride (ChCl)
A $(OEG)_1$	71.01	3.36	C_α−H_α in β-O-4' (OEG) aryl-alkyl ether with EG incorporation at the α position
B_γ	71.11	3.81	C_γ−H_γ in resinol substructures (B)
B_γ	71.28	4.24	C_γ−H_γ in resinol substructures (B)
	77.28	3.02	Carbohydrates C_2-C_4 region
A_α (OEG)	80.97	4.61	C_α−H_α in β-O-4' (OEG) aryl-alkyl ether with EG incorporation at the α position
A_β (OH)	82.63	4.42	C_β−H_β in β-O-4'(OH) aryl-alkyl ether
A_β (OEG)	85.13	4.26	C_β−H_β in β-O-4' (OEG) aryl-alkyl ether with EG incorporation at the β position
B_α	85.44	4.68	C_α−H_α in resinol substructures (B)
$S_{2.6}$	104.46	6.56	$C_{2.6}$–$H_{2.6}$ in syringyl units (S)
$S_{2.6}$	105.23	6.75	$C_{2.6}$–$H_{2.6}$ in syringyl units (S)
$S'_{2.6}$	106.40	7.38	$C_{2.6}$–$H_{2.6}$, oxidized (C_α=O) S units (S’)
G_2	111.00	7.03	C_2−H_2 in guaiacyl units (G)
G'_2	111.00	7.31	C_2−H_2, oxidized (H–C_α=O) G units (G’)
G'''_2	113.70	7.44	C_2−H_2, oxidized (HO–C_α=O) G units (G''')
G_5	115.53	6.49	C_5–H_5 in guaiacyl units (G)
G_5	115.85	6.98	C_5–H_5 in guaiacyl units (G)
G_6	119.78	6.89	C_6−H_6 in guaiacyl units (G)
OA	129.90	5.34	C-H in double bond of oleic acid

Table S5. Molecular weight (M_w), molecular number (M_n) and polydispersity index (PI) of the LIG-ES extraction with ChCl:pTSA:EG (80 ºC, 4 h) and Kraft lignin by GPC.

	M_w (g·mol^{-1})	**M_n (g·mol^{-1})**	**PI**
[a]Kraft lignin	1520	1345	1.13
LIG-ES	9167	1608	5.70

[a]Dias et al.[52]

4. References

(16) Erdocia, X.; Prado, R.; Corcuera, M. Á.; Labidi, J. Effect of Different Organosolv Treatments on the Structure and Properties of Olive Tree Pruning Lignin. *J. Ind. Eng. Chem.* **2014**, *20*, 1103–1108. https://doi.org/10.1016/j.jiec.2013.06.048.

(17) Eugenio, M. E.; Martín-Sampedro, R.; Santos, J. I.; Wicklein, B.; Ibarra, D. Chemical, Thermal and Antioxidant Properties of Lignins Solubilized during Soda/AQ Pulping of Orange and Olive Tree Pruning Residues. *Molecules* **2021**, *26* (3819).

(18) Santos, J. I.; Martín-Sampedro, R.; Fillat, Ú.; Oliva, J. M.; Negro, M. J.; Ballesteros, M.; Eugenio, M. E.; Ibarra, D. Evaluating Lignin-Rich Residues from Biochemical Ethanol Production of Wheat Straw and Olive Tree Pruning by FTIR and 2D-NMR. *Int. J. Polym. Sci.* **2015**, *2015*. https://doi.org/10.1155/2015/314891.

(19) Santos, J. I.; Fillat, Ú.; Martín-Sampedro, R.; Eugenio, M. E.; Negro, M. J.; Ballesteros, I.; Rodríguez, A.; Ibarra, D. Evaluation of Lignins from Side-Streams Generated in an Olive Tree Pruning-Based Biorefinery: Bioethanol Production and Alkaline Pulping. *Int. J. Biol. Macromol.* **2017**, *105*, 238–251. https://doi.org/10.1016/j.ijbiomac.2017.07.030.

(23) Sosa, F. H. B.; Abranches, D. O.; Da Costa Lopes, A. M.; Coutinho, J. A. P.; Da Costa, M. C. Kraft Lignin Solubility and Its Chemical Modification in Deep Eutectic Solvents. *ACS Sustain. Chem. Eng.* **2020**, *8*, 18577–18589. https://doi.org/10.1021/acssuschemeng.0c06655.

(58) Dias, R. M.; da Costa Lopes, A. M.; Silvestre, A. J. D.; Coutinho, J. A. P.; da Costa, M. C. Uncovering the Potentialities of Protic Ionic Liquids Based on Alkanolammonium and Carboxylate Ions and Their Aqueous Solutions as Non-Derivatizing Solvents of Kraft

Lignin. *Ind. Crops Prod.* **2020**, *143*, (111866). https://doi.org/10.1016/j.indcrop.2019.111866.