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Lignin as bio-based raw material: structural investigation for a better valorization

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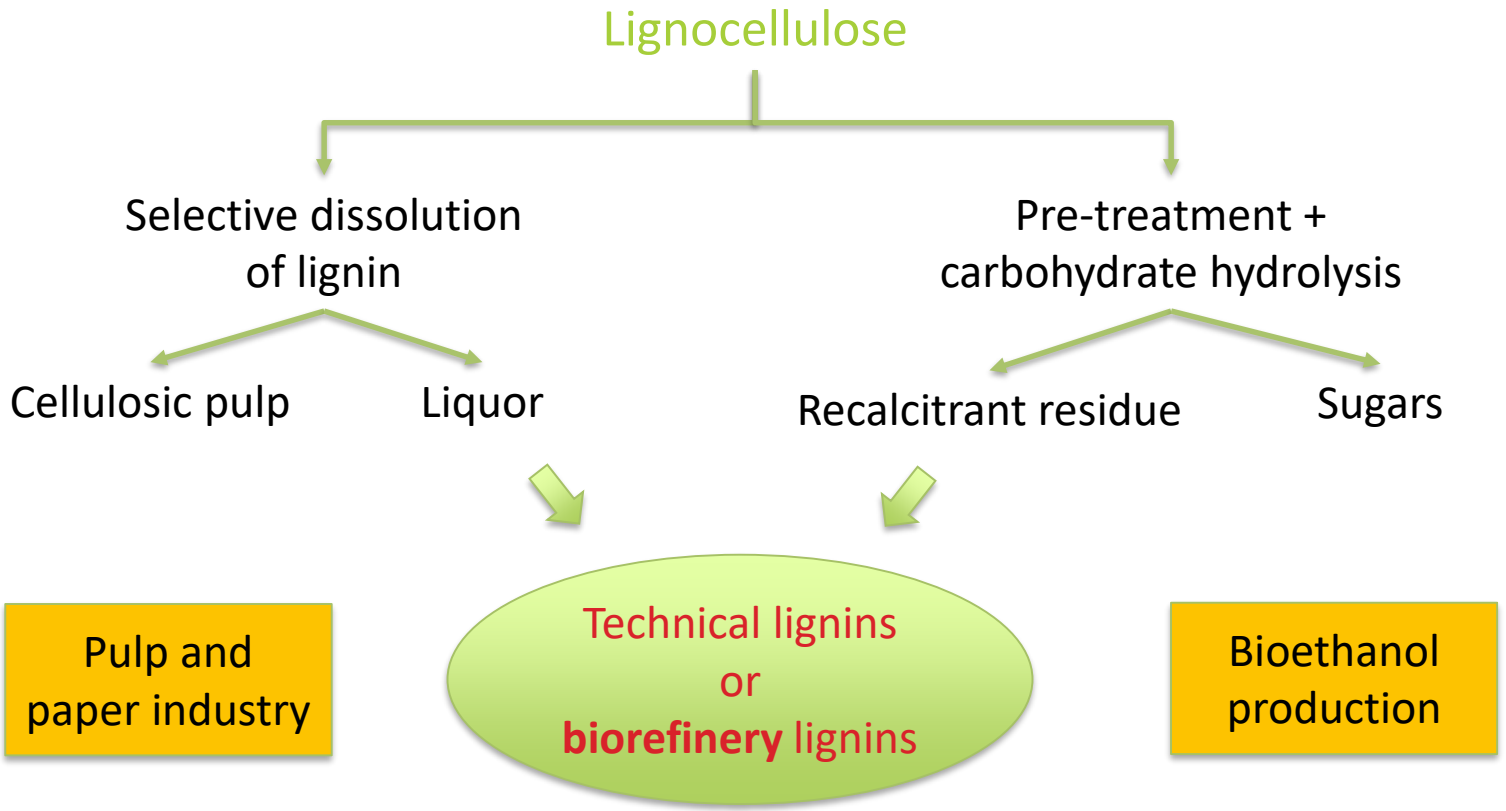
Outline

- Technical lignins and their markets
- A strategy to manage variability and exploit heterogeneity
- Activity of a lignin ethyl acetate extract

What are technical lignins ?

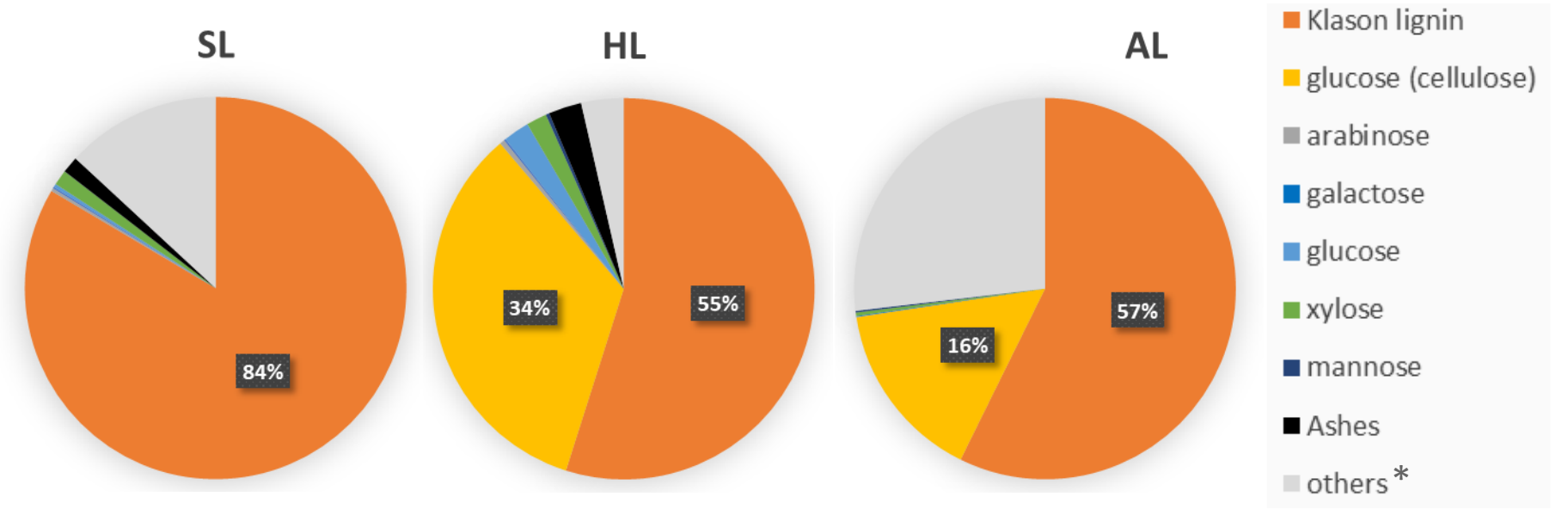
(Vishatal and Kraslowaski, 2011; Constant et al. 2016; Takkellapati, 2018; Balaksin et al 2021)

By-products of lignocellulose transformation



Heterogeneous and variable raw materials

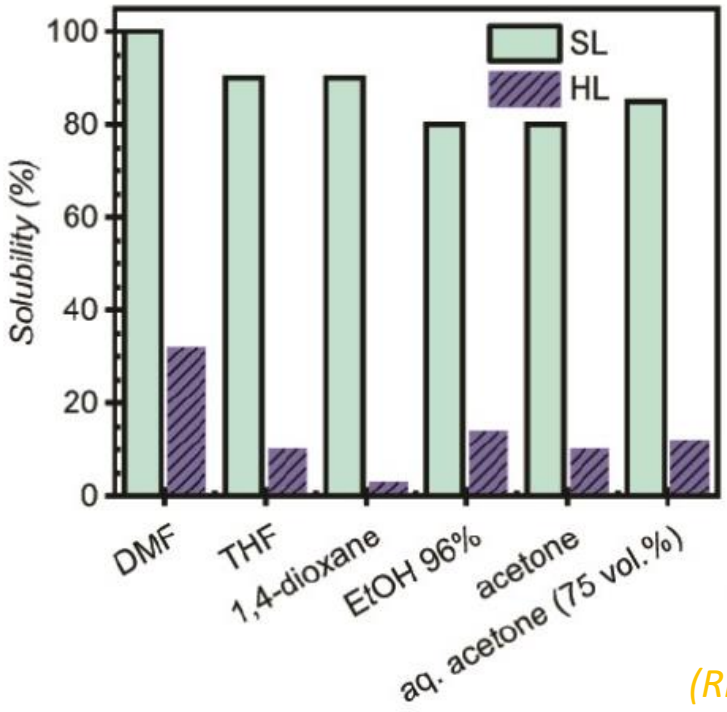
- Composition of 3 types of biorefinery lignins (Zelcor project)
  - SL=grass soda lignin (Protobind 1000, Green value LLC)
  - HL=wheat straw residue from ethanol production (Futurol project)
  - AL= softwood acidic lignin (Dawn technology)



* acid soluble lignin, uronic acids, extractives, proteins

This project has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation program under grant agreement No 720303

- Solubility of some technical lignins in aqueous and organic solvents
  - SL=soda lignin
  - HL=residue from ethanol production



(Rivière et al., 2021)

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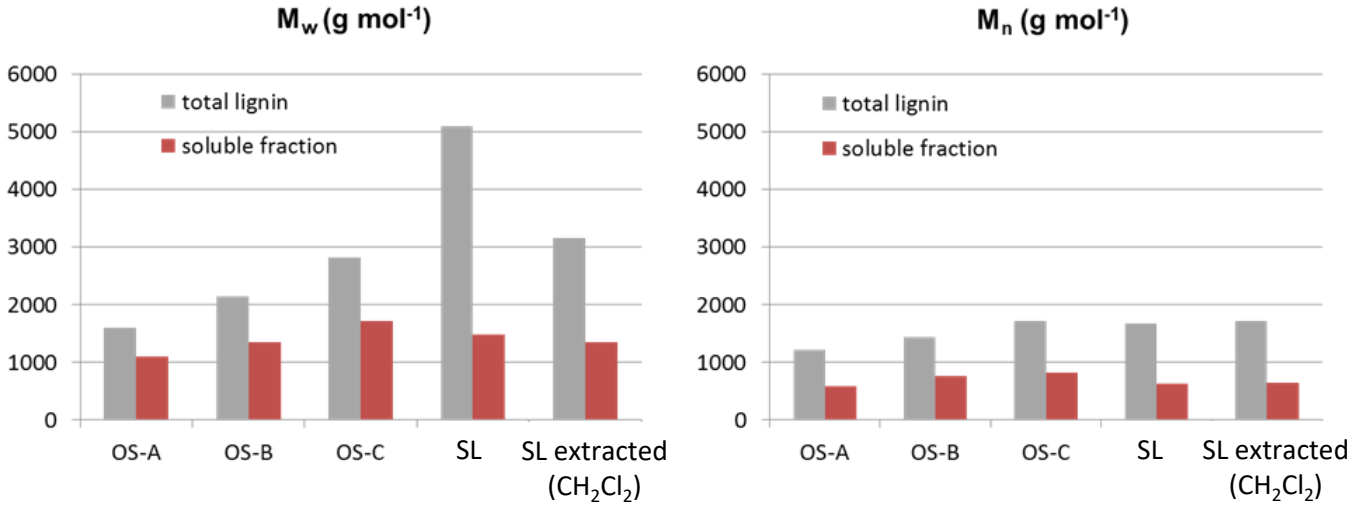
Particularity of organosolv lignins

- High purity (up to 95% lignin content) ⇨ advantage for organic synthesis
- Solubility in common organic solvents (e.g. aqueous ethanol, acetone)
- Hydrophobicity (non soluble in water)
- Low molar masses (Mw < 5000 g.mol^{-1}) and polydispersity (~2) ⇨ possibility to get standardized extracts



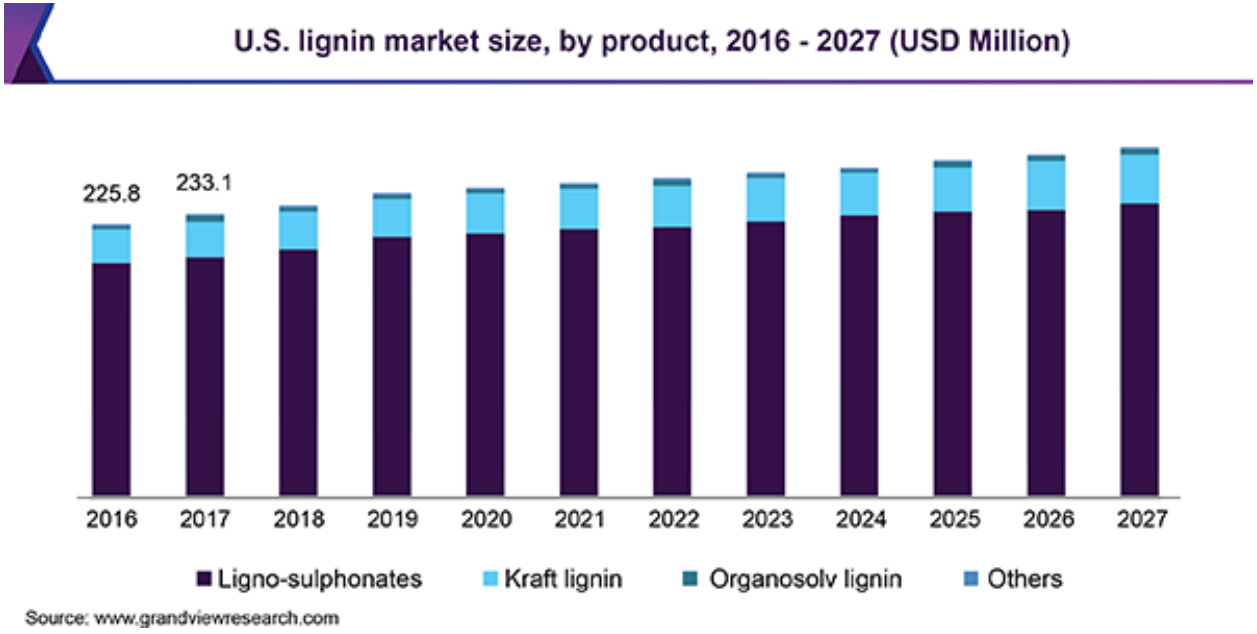
Average molar masses of lignin samples and their ethanol extracts

(Aguié et al., 2015)

Interest and market for lignins

A 2% global annual market increase forecast

- Grand View Research survey (2020) - *Lignin Market Size, Share & Trends Analysis Report By Product (Ligno-Sulphonates, Kraft, Organosolv), By Application (Macromolecule, Aromatic), By Region, And Segment Forecasts, 2020 – 2027*



Existing applications for 20 years

Specialty cellulose	Lignin	Vanillin	Bioethanol
Construction materials	Concrete additive	Food	Car care
Cosmetics	Animal feed	Perfumes	Paint/varnish
Food	Dyestuff	Pharmaceuticals	Pharmaceutical industry
Tablets	Batteries		Bio fuel
Textiles	Briquetting		
Filters	Mining		
Paint/varnish	Soil conditioning		

https://www.borregaard.com/Products-Solutions

Lignin in fuel oil

Lignin fuel in lime kilns

Lignin pellets

Dispersants

Kaolin/Water

Lignin to carbon fibres

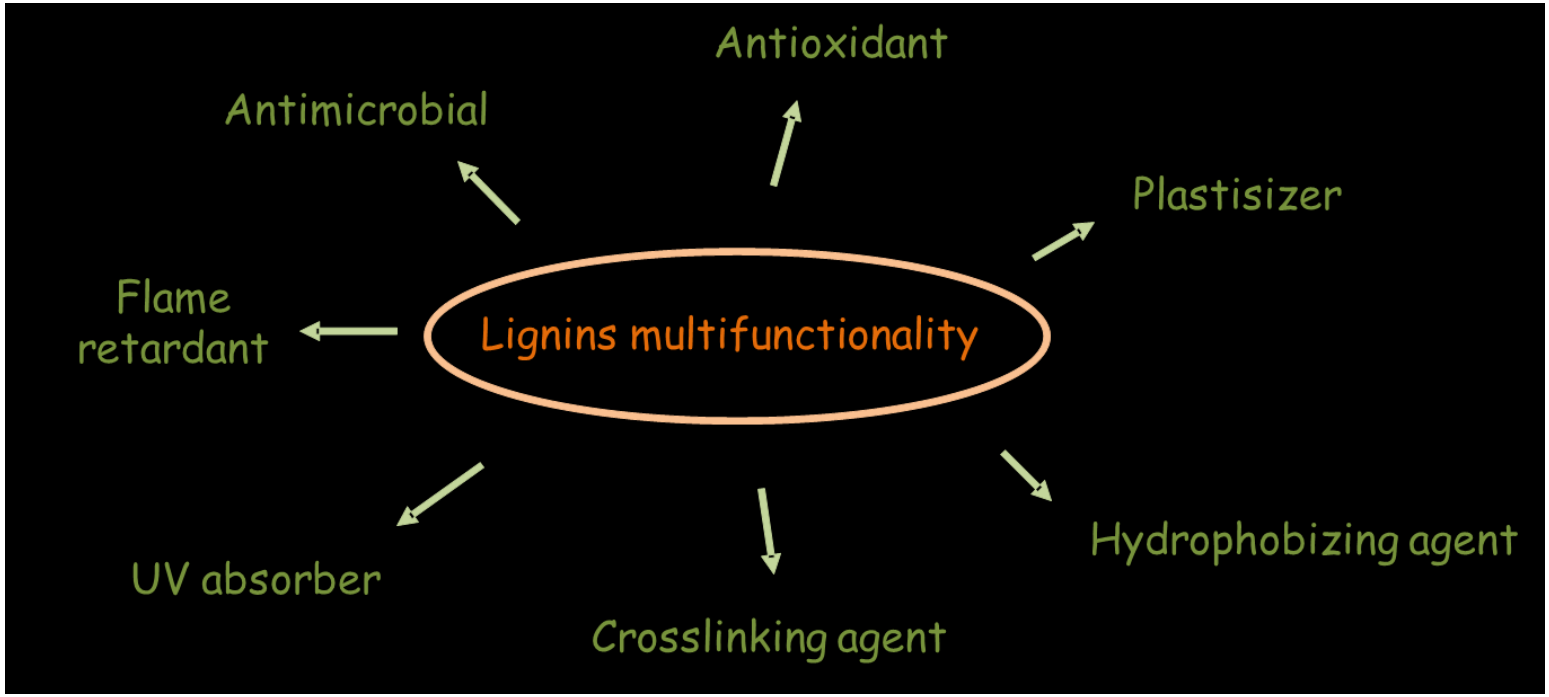
Spun lignin fibres

Other applications

- **Binders**
- **Benzene/Phenols**
- **Activated carbon**

INNVENTIA, "Biorefinery within the Pulp & Paper sector", 2009.

Multifunctionality still unexploited



⇨ Potential applications as bio-based additives in plastic films for mulching, active packaging or biomedical materials

Bottleneck: need to characterize lignin structure and manage its variability

A strategy to manage variability and exploit heterogeneity

Integrated cascading approach

- Combined solvent extraction - depolymerization - bioconversion

Technical lignin
= phenolics
+ carbohydrates
+ ashes
+ proteins, …

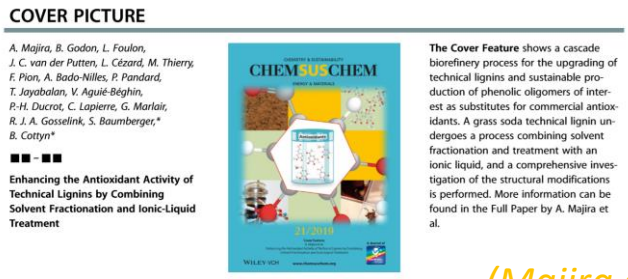
1st stage extraction (ethyl acetate, EA)

Functional phenolic extract (32%)
✓ Soluble, biologically active, antioxidant
✓ Standardised Mn and Mw (600-800 g.mol^{-1})

2nd stage extraction (ethanol)

Lignin fraction suitable for depolymerization (34%)
✓ Less recondensations

Residue suitable for bioconversion (34%)
✓ No toxic phenolics
✓ Plant cell-wall polymers


COVER PICTURE

A. Majira, B. Godon, L. Foulon, J. C. van der Putten, L. Cézard, M. Thierry, F. Pion, A. Bado-Nilles, P. Pandard, T. Jayabalan, V. Aguié-Béghin, P.-H. Ducrot, C. Lapierre, G. Marlair, R. J. A. Gosselink, S. Baumberger,* B. Cottyn*

■■ - ■■

Enhancing the Antioxidant Activity of Technical Lignins by Combining Solvent Fractionation and Ionic-Liquid Treatment

The Cover Feature shows a cascade biorefinery process for the upgrading of technical lignins and sustainable production of phenolic oligomers of interest as substitutes for commercial antioxidants. A grass soda technical lignin undergoes a process combining solvent fractionation and treatment with an ionic liquid, and a comprehensive investigation of the structural modifications is performed. More information can be found in the Full Paper by A. Majira et al.

(Majira et al., 2019)

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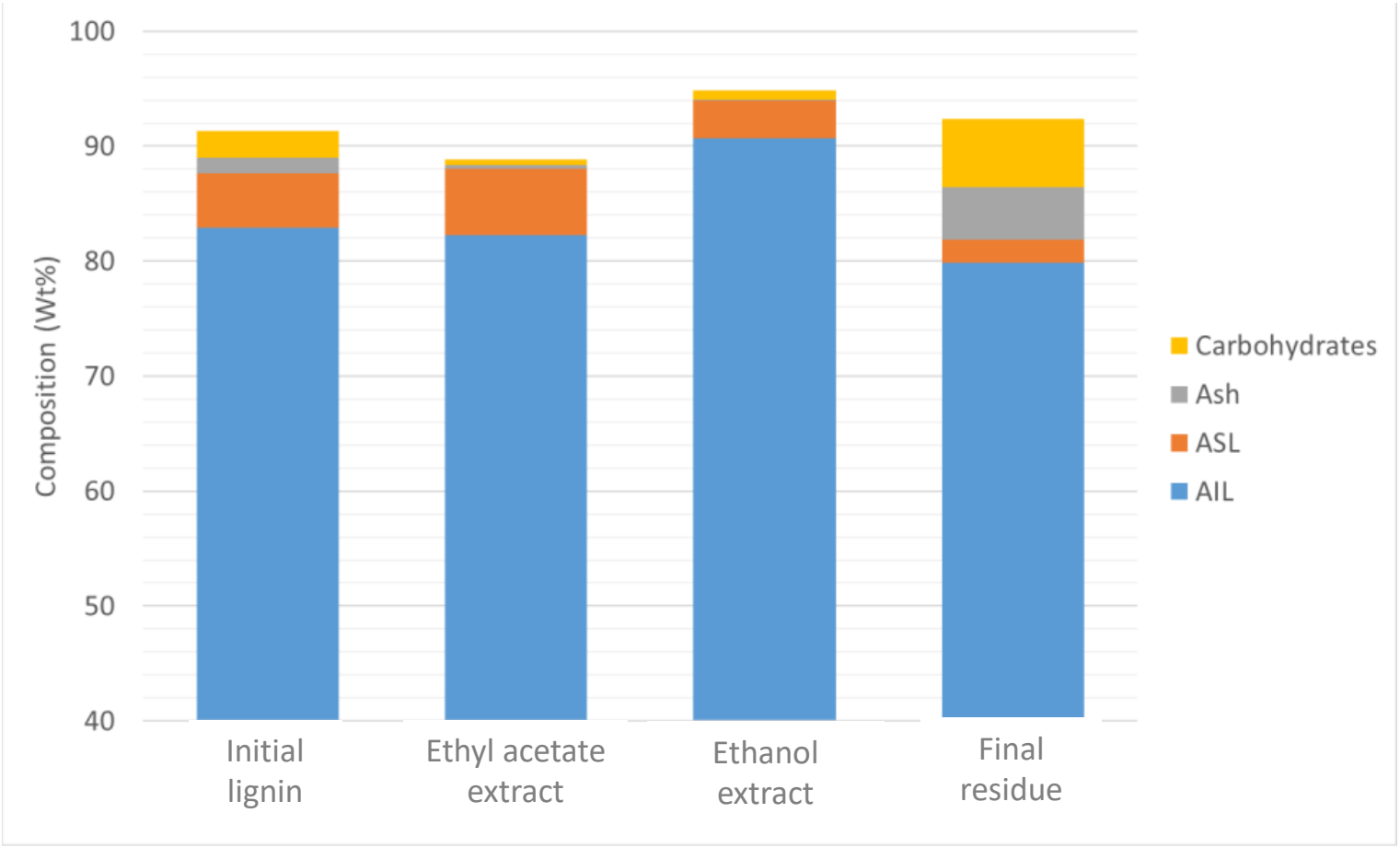
Characterization of the fractions (soda lignin)

- Composition



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Characterization of the fractions (soda lignin)

- Molar mass distribution (Mn and Mw, $g.mol^{-1}$)
  - *Ethyl acetate extract: Mn=1015; Mw=1260 ↔ LMM oligomers (DP=5-6)*
  - *Ethanol extract: Mn= 1000; Mw=2500 ↔ HMM oligomers (DP=5-12)*
  - *Non soluble residue: Mw and Mn non determined ↔ polymers*

- Monomer composition of the initial lignin (extracted by EA)

Compound number	Compound name	*c* ($mg\ g^{-1}$) [a]
		HPLC[a]
Major compounds		
1	vanillin	1.99
2	syringaldehyde	2.27
3	acetosyringone	4.76
4	*p*-coumaric acid	1.13
5	ferulic acid	1.09
Minor compounds		2.46
Total compounds		13.70

[a] HPLC-MS analysis of extracts (10 mg sample extracted by 1 mL water, PH 3, ambient T°C, 2 h under stirring) purified with speak cartridge using MeOH as eluent; determination based on *o*-coumaric acid internal standard; determination in duplicate (error < 3%).

Majira *et al.*, 2019

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Characterization of the fractions (soda lignin)

- Mapping of functional properties

Deliverable D5.3 Mapping of functional properties

Score	Legend
5	High functionality
4	
3	Medium functionality
2	
1	Low functionality

Sample	Processing	Zelcor code	Antioxidant in PE: OIT (min) 5wt%	Antioxidant in PE: OIT (min) 2 wt%	Antioxidant in solution: 1/CE50 (ml/mg) before IL treatment	1/CE50 (ml/mg) after IL treatment*	1/CE50 (ml/mg) after IL treatment**	Radical formation protection: ORAC	Radical formation protection: ROS reduction (%)	Antimicrobial activity: Growth factor reduction (Log) In PE, S. Aureus	In PE, E. Coli	In cellulose film, S. Aureus	In cellulose film, E. Coli
Soda lignin	As received	GV03	57.3	50.8				3313	30.50	1.8	0.11		
Soda lignin	Fractionated	GV03FB01	80.2	63.5	3.7	9.1	2.4	4416	0	3.74	0.16		
		GV03FB02			3.7	9.1	6.7	3043	0				
		GV03FB03			2.6	3.0	5.9	1394	0				
		GV03FB04			2.6			0	0				
		GV03FB05											
	Water insoluble	GV03FA01			2.5							2	1
	Water soluble	GV03FA02			3.7								
Nanoparticles	CLPs water soluble	GV03CLP			3.3								
Distillation residue	Dried	FU01	31	16.2						0	0		
HUMIN		AV01						664	52				
References													
Coniferyl alcohol					4.5								
p-coumaryl alcohol					4.0								
Ferulic acid					4.8								
Indulin					2.6								

Sample	Zelcor code	Anti-insect activity: 1/N-insect In PE	Solubility: wt% in THF/water	Optical properties: UV Extinction coeff. ($L\,g^{-1}\,cm^{-1}$) in aqueous dioxan	UV Extinction coeff. in aqueous ethanol	UV Extinction coeff. in water	Film transmitt. (%) 280 nm	Film transmitt. (%) 700 nm	UVB protection	DNA protection	cytokin reduction	sirtuin expression	anti-inflammation	spf activity
Soda lignin	GV03	0.24	100						30					
Soda lignin	GV03FB01	0.31	104		24.9		2	86						
	GV03FB02		102		24.8		3	88						
	GV03FB03		99	23.5			2	87						
	GV03FB04		105	24.9			2	89						
	GV03FB05		12											
	GV03FA01			24.2	23.5		0	87						
	GV03FA02													
Nanoparticles	GV03CLP				24.6	32.6	1	90						
Distillation residue	FU01	0.15	35											
HUMIN	AV01								100	-69	-63	64	-40	58

* EtOAC extract

*EtOAC extraction residue

Score	Legend
5	High functionality
4	
3	Medium functionality
2	
1	Low functionality

ZELCOR
Bio-based Industries Consortium
Horizon 2020 European Union Funding for Research & Innovation

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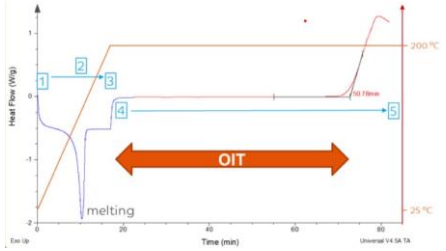
Activity of the EA extract

- Antioxidant properties
  - In HDPE: Oxidation Induction Time (OIT, min)

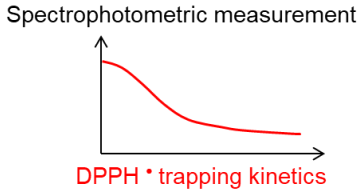
Sample	OIT at 5 wt%	OIT at 2 wt%
PE - reference	1.1 min (0 wt%)	
PE – HL	31.0 min	16.2 min
PE – initial SL	57.3 min	50.8 min
PE – EA extract	80.2 min	63.5 min



(Vachon et al., 2020)

  - In solution: DPPH scavenging test (EC50, $mg.mL^{-1}$)

Sample	EC_{50} (before IL treatment)	EC_{50} (after IL treatment)
Intial SL	0.40	
EA extract	0.27	0.11



(Majira et al., 2019)

  - Radical formation protection: Oxygen Radical Absorbance Capacity (ORAC)
    - AAPH test against a fluorophore

Sample	µmol Trolox eq. g^{-1}	% Trolox activity
Initial SL	3313	83
EA extract	4116	103

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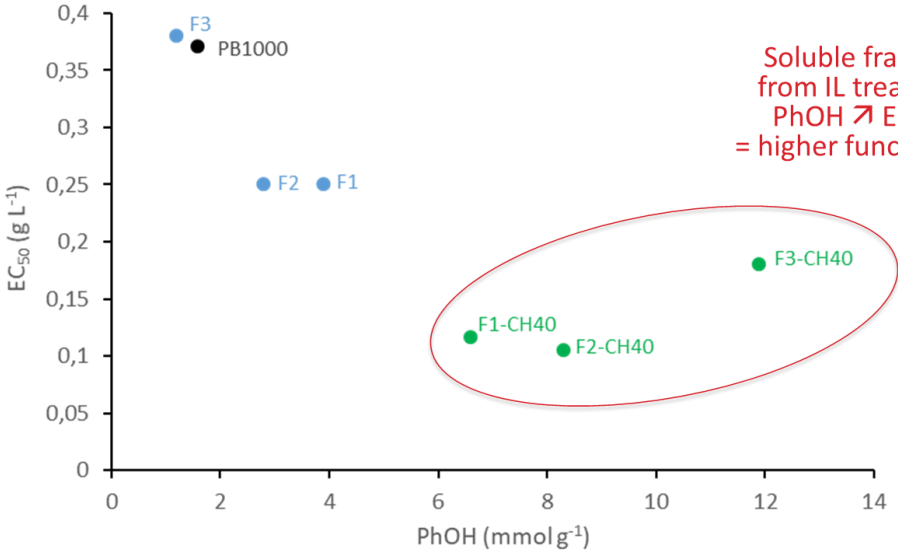
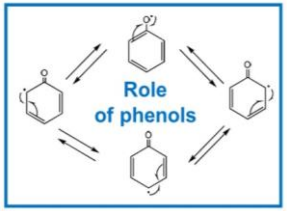
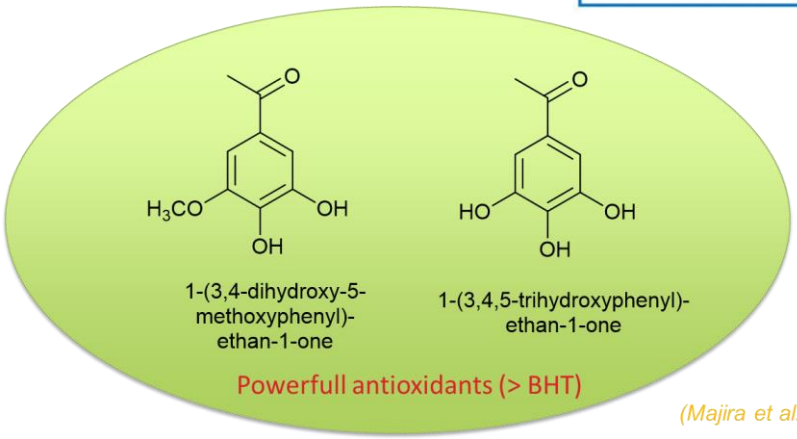
- Correlation between phenol groups content and antioxidant properties

(2)		HL	Initial SL	EA extract
TOTAL OH	[mmol/kg]	5.7	5.8	6.2
CARBOXYLIC OH	[mmol/kg]	0.2	0.9	1.1
ALIPHATIC OH	[mmol/kg]	4.5	1.5	0.9
AROMATIC OH	[mmol/kg]	1.0	3.4	4.2



Soluble fractions from IL treatment
PhOH ↗ EC_{50} ↘
= higher functionality





1-(3,4-dihydroxy-5-methoxyphenyl)-ethan-1-one

1-(3,4,5-trihydroxyphenyl)-ethan-1-one

Powerfull antioxidants (> BHT)

(Majira et al., 2019)

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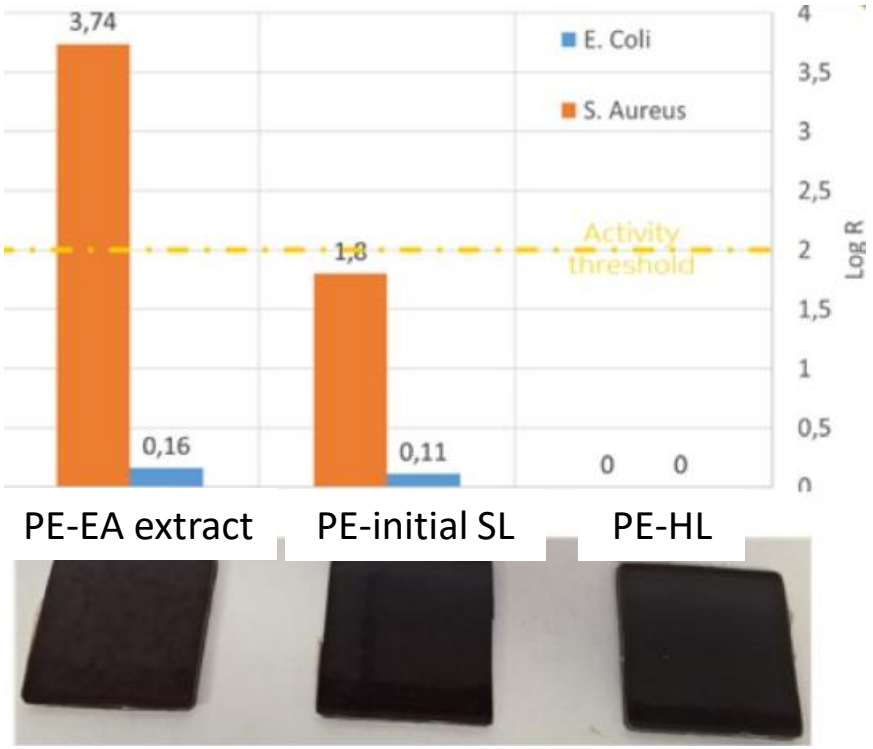
- Antimicrobial properties
  - In HDPE: Growth factor reduction (Log) after 24h incubation
    - Against *S. Aureus* (gram+)
    - Against *E. Coli* (gram-)



(Vachon et al., 2020)

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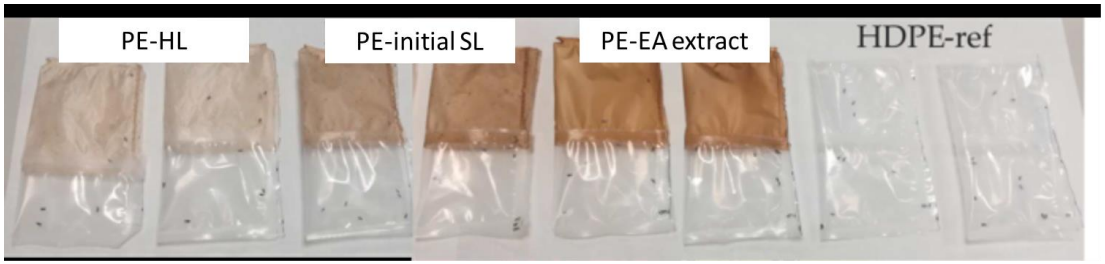
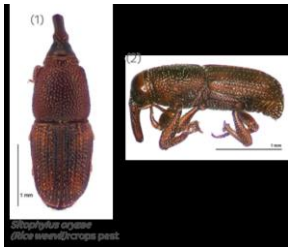
- Anti-insect properties
  - In HDPE: invader test against *Sitophylus oryzae*





Sample code	N-insects	Effect
HDPE-ref	4,2	No repellence (4-6)
HDPE – HL (2wt%)	6,7	Attractant (>6)
HDPE – Initial SL (2wt%)	4,6	No repellence (4-6)
HDPE – EA extract (2wt%)	**3,2**	**Repellent (<4)**

(Vachon et al., 2020)

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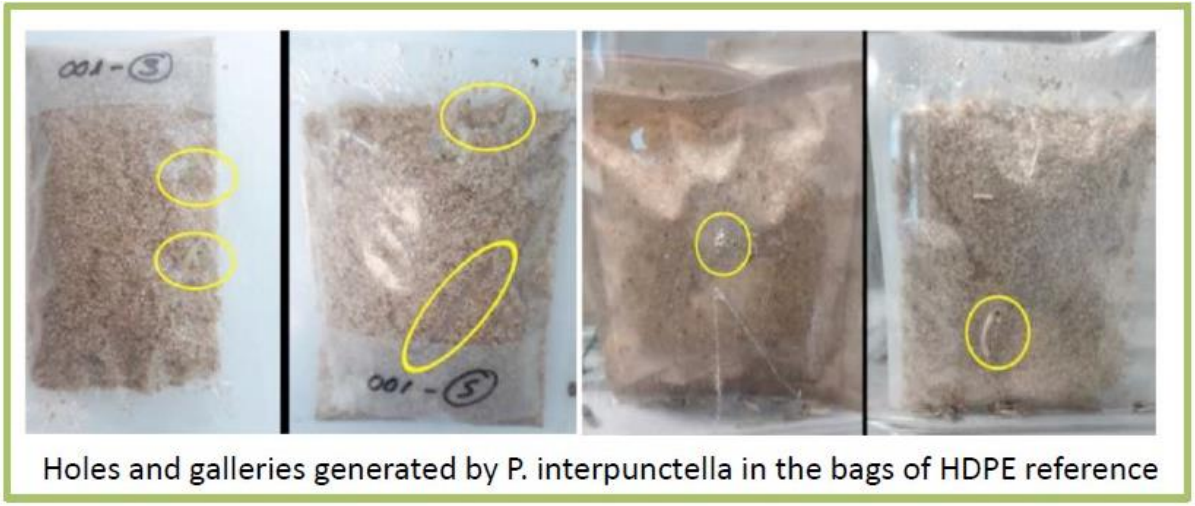
- Anti-insect properties
  - In HDPE: penetrator test against *Plodia Interpunctella*



Holes and galleries generated by P. interpunctella in the bags of HDPE reference

Material	#small larvae	#larger larvae	#Pupals	#adults	Total
PE-ref	4,5 ± 1,2	2,4 ± 1,1	3,6 ± 1,6	1,6 ± 1,2	12,1 ± 4,4
PE-HL	0,6 ± 0,4	1,0 ± 0,7	1,5 ± 0,8	0	3,1 ± 1,6
PE-initial SL	3,9 ± 0,6	2,5 ± 0,6	0,8 ± 0,4	0	7,1 ± 1,0
PE-EA extract	0	0	0	0	0

(Vachon et al., 2020)

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Conclusions

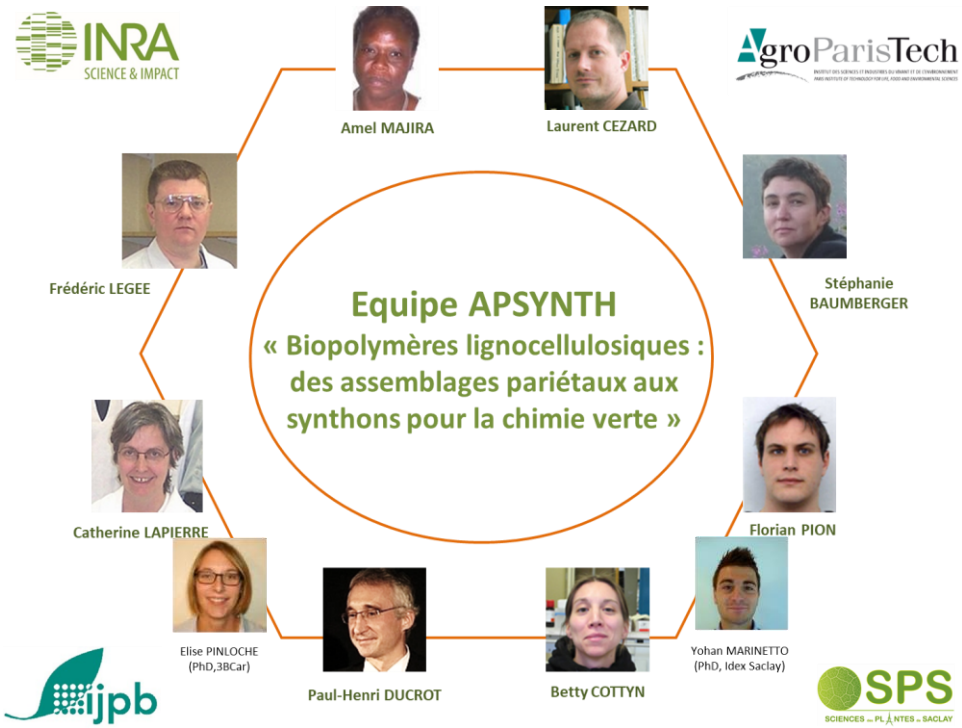
- Necessity to take into account the ethyl acetate extractible fraction for lignin valorization
  - Favor side-chain reaction during depolymerizing treatments
  - Negative impact on lignin bioconversion by insects
  - Source of valuable molecules (multifonctional properties)

- Industrial processes to be developed for the product of such a fraction

- Advantage of grass soda lignins
  - Cleavage of ester bonds with release of *p*-hydoxycinnamic acids
  - Selective dissolution of compounds with higher phenol contents

Acknowledgements

- Zelcor project consortium
- Université de Versailles St-Quentin-en-Yvelines
- Institut de Chimie Moléculaire et des Matériaux d'Orsay
- UMR1318 IJPB Apsynth team and « Observatoire du végétal »



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