Confidential

Biorefinery lignins use as antioxidants: radical scavenging properties in packaging and food model matrices

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Biorefinery lignins use as antioxidants: radical scavenging properties in packaging and food model matrices

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Economical context

- A growing market for antioxidants

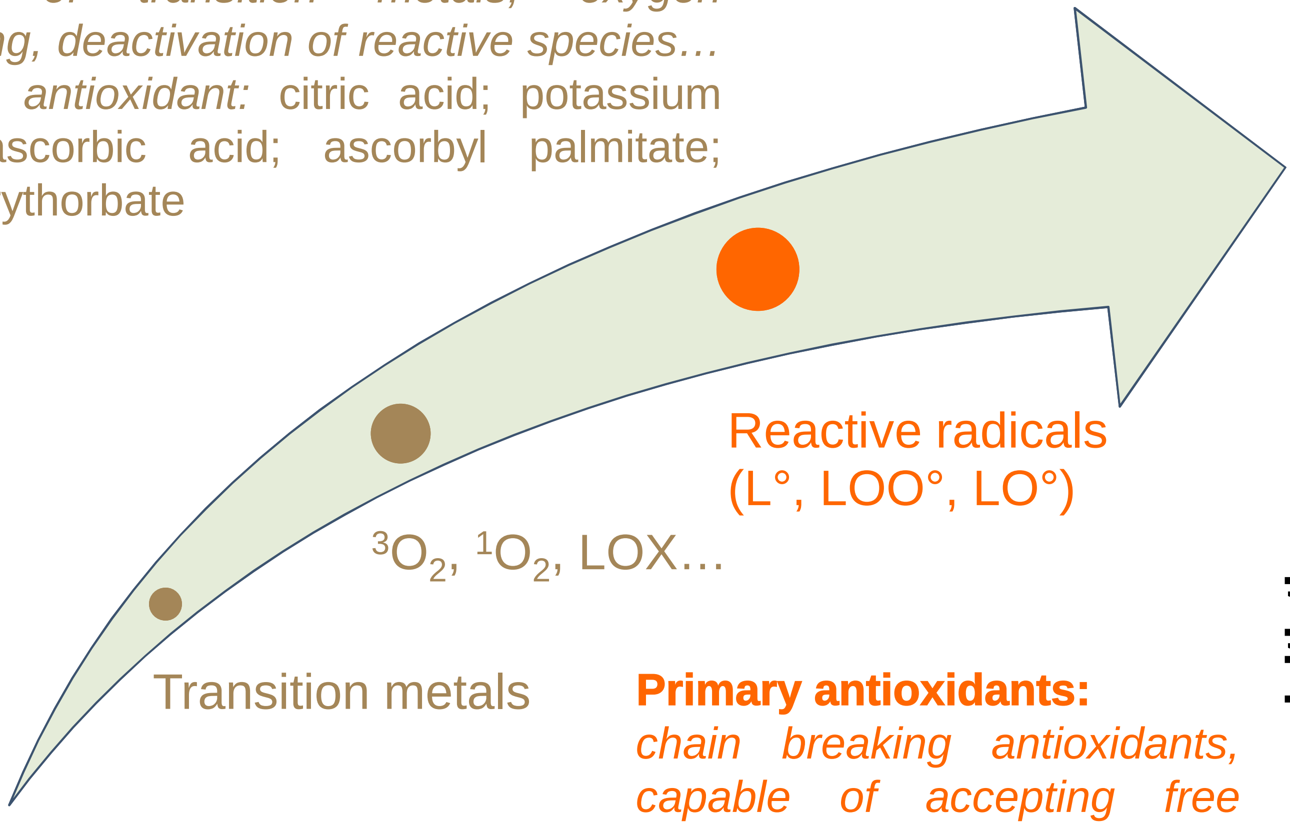
  - global market of USD 2.25 billion in 2015
  - expected 5-6 % revenue growth per year between 2015 and 2020
  - major consumers : rubbers, plastics and fuels
  - other uses : lubricants, adhesives, cosmetics, food

- An expected increase of the demand in natural antioxidant for food and personal care products

Source: Zion Research Analysis, 2015. “Antioxidants (Natural, and Synthetic) Market for Pharmaceuticals, Food & Beverages Sector, Feed Additives, Cosmetics Industry, and Other Applications: Global Industry Perspective, Comprehensive Analysis, and Forecast, 2014 – 2020”

Antioxidant mechanisms

Secondary antioxidants:
chelation of transition metals, oxygen scavenging, deactivation of reactive species…
e.g. food antioxidant: citric acid; potassium citrate; ascorbic acid; ascorbyl palmitate; sodium erythorbate



Oxidation products
(aldehydes, ketones, polymers, …)

Reactive radicals
(L°, LOO°, LO°)

$^3O_2$, $^1O_2$, LOX…

Transition metals

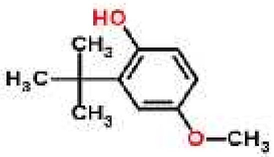
Unsaturated fatty acids
or polymers

Primary antioxidants:
chain breaking antioxidants, capable of accepting free radicals so that they can delay the initiation step or interrupt the propagation step.

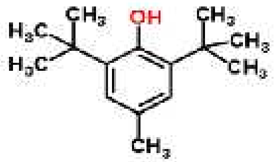
OXIDATION

initiation	LH → L• + H•
	LH + $M^{(n+1)+}$ → L• + H^+ + M^+
	LOOH → LO• + OH•
	LOOH + M^{n+} → LO• + OH• + $M^{(n+1)+}$
	LOOH + $M^{(n+1)+}$ → LOO• + H• + M^{n+}
propagation	L• + $^3O_2$ → LOO•
	LOO• + LH → LOOH + L•
	LOO• + LOOH → LOOH + LOO•
	LO• + LH → LOH + L•
	LO• + LOOH → LOH + LOO•

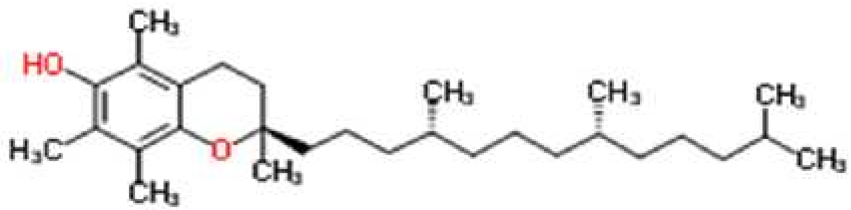
Commercial antioxidants



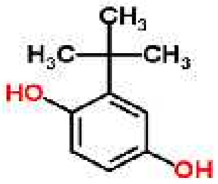
Butylhydroxyanisole BHA (E320)



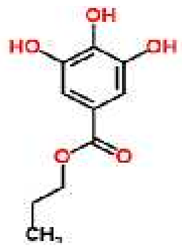
Butylhydroxytoluene BHT (E321)



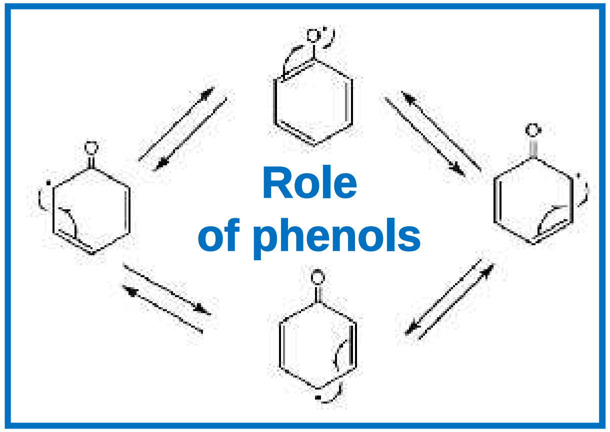
Tocopherol extracts (E306), α-Toc (E307), γ-Toc (E308), δ-Toc (E309)

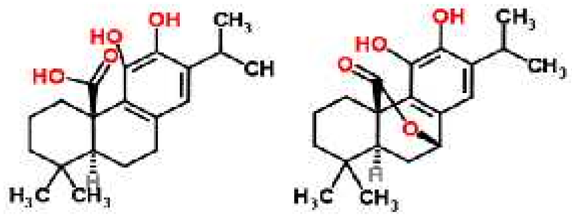


Tert-butylhydroquinone TBHQ (E319)

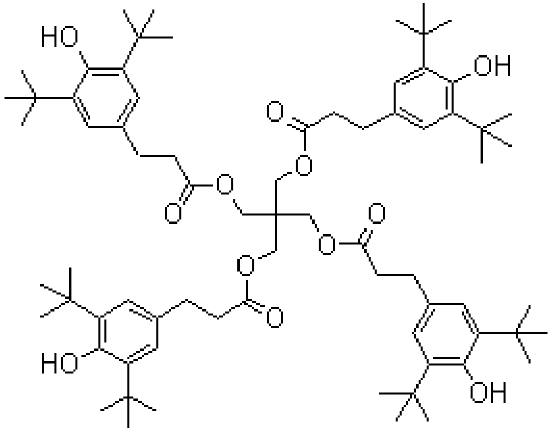


Propyl gallate (E310), …

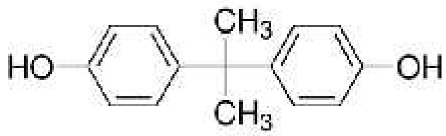




Rosemary extract (carnosic acid & carnosol) (E392)



Irganox



Bisphenol A

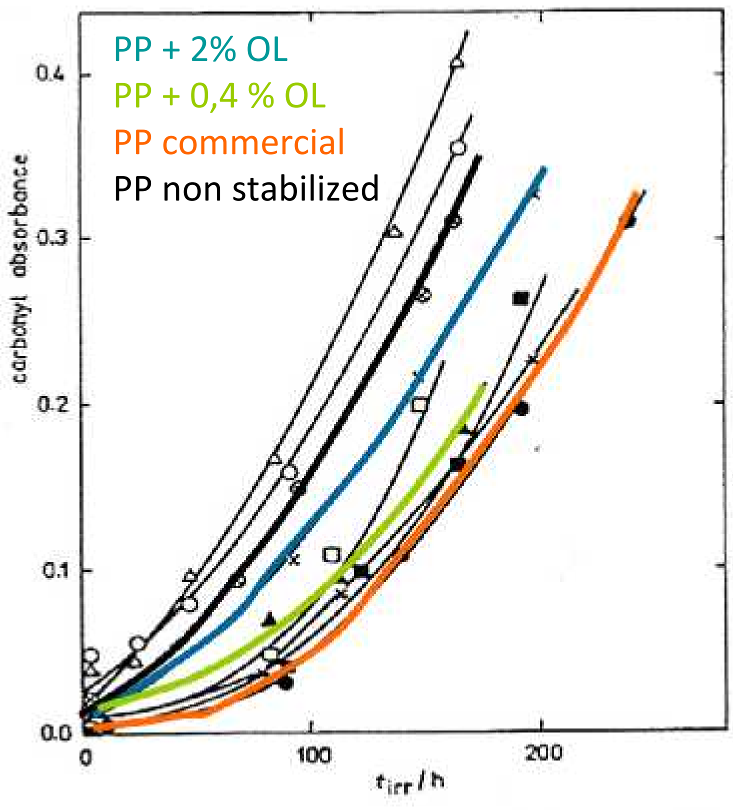
Regulation (EC) N° 1333/2008 - Food additives

Lignins as biobased antioxidants

Polymer protection

PP photo-oxidation (*Kosikova et al. 1993*)

PU thermo-oxidation (*Arshanitsa et al. 2013*)

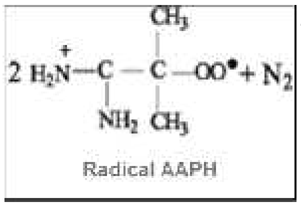


⇨ Thermoplastics, elastomers

Human cell protection

Oxidative stress of erythrocytes

(Vinardell et al. 2008)



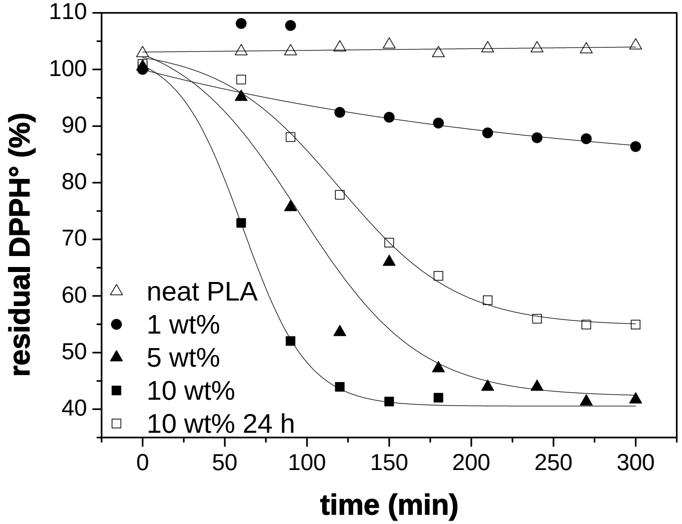
Product	EC50 (µg/ml)
Alkali lignin	44,9 ± 6.7
Lignosulfonates	133,6 ± 9.0
Kraft lignin	85,9 ± 4.6
Steam explosion	74,6 ± 1.0
(−)-Epicatechin	42,27 ± 3.0

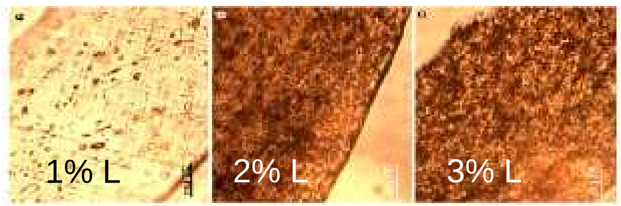
⇨ Cosmetics

Scavenging activity

Radical trapping by PLA-lignin films

(Domenek et al. 2013)





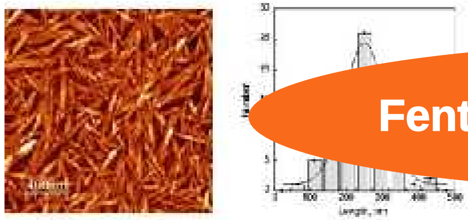
Laccase-catalyzed O_2 scavenging by starch-lignin coatings

(Johansson et al. 2014)

⇨ Active packaging

Lignin Multi-functionality

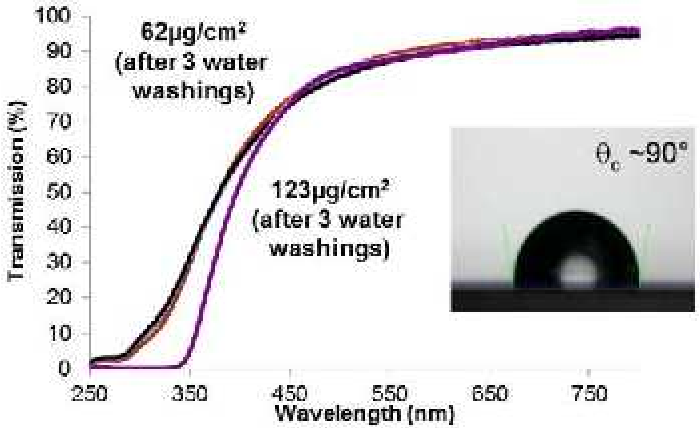
Cellulose – lignin nanocomposites *(Hambardzumyan et al 2012)*

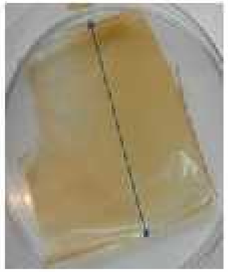


Fenton reagent ↔cross-linking

Cellulose nanocristals
+
Industrial lignins (17%)

Ultra-thin films on quartz (thickness 3-4 µm)





Self supported films (thickness 25-30 µm

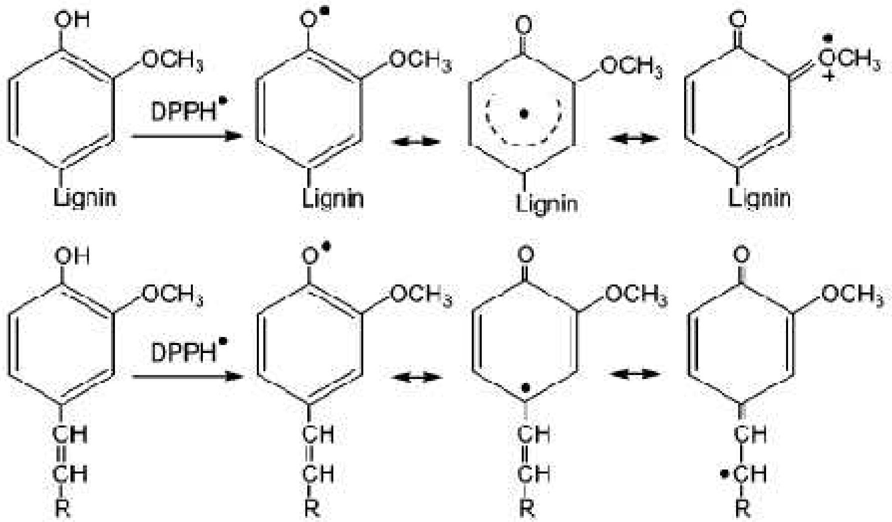
⇩

Medical antioxidant carriers ;
active packaging

- ✓ Antioxidant
- ✓ Hydrophobicity
- ✓ Anti-UV
- ✓ Cross-linking capacity

Influence of lignin structure

Role of phenolic hindered hydroxyl groups

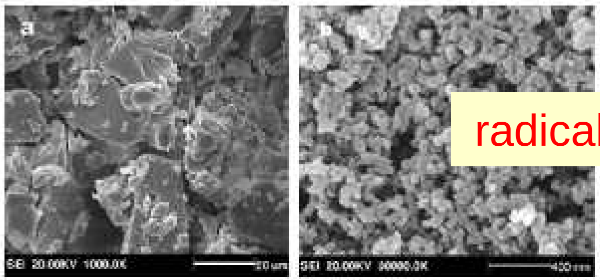


(Dizhbite et al 2004)

Influence of physical state

⇔ correlation with lignin solubility

(nanoscale lignins; El Hage et al. 2012)



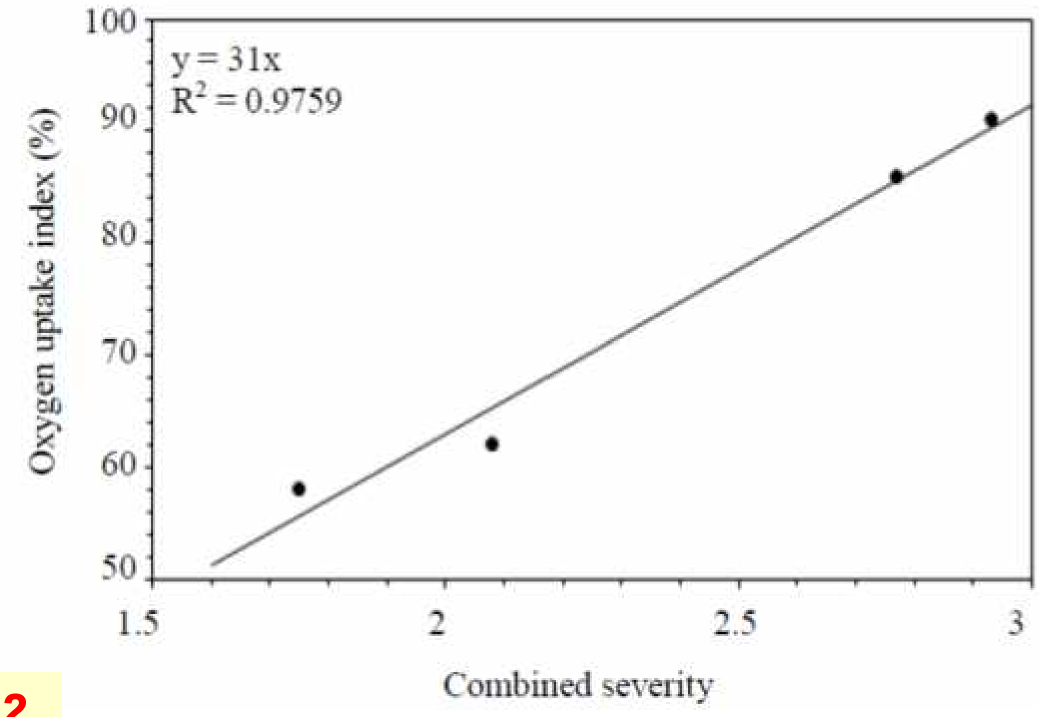
radical scavenging power **x 12**

SEM image of non-nanoscale lignin and nanoscale lignin recovered by OS lignin dissolution in acetone and precipitation in of supercritical CO_2 *(El Hage et al. 2012)*

Positive effect of delignification treatment severity

⇔ correlation with phenol content and molar masses

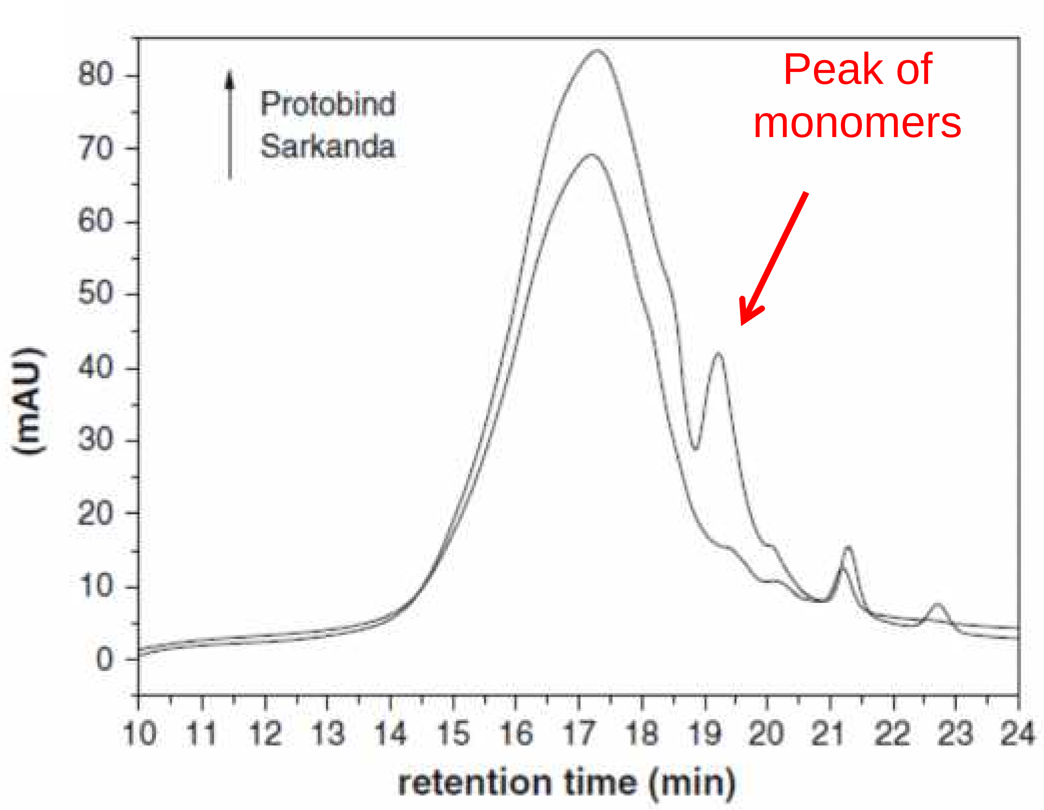
(ethanol delignification of hybrid poplar; Pan et al. 2006 and Miscanthus x giganteus; El Hage et al. 2012)



Oxygen uptake during the oxidation of methyl linoleate induced by 2,2'-azobisisobutyronitrile (AIBN) *(El Hage et al. 2012)*

Role of phenolic extractives

Comparison of alkali Sarkanda and Protobind lignins

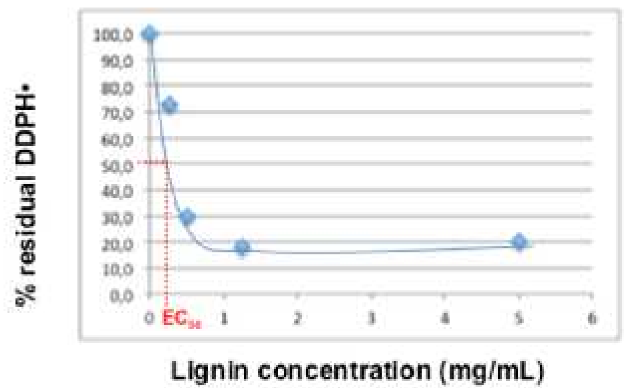


Size-exclusion chormatogram of lignins after acetylation (PL-GEL mixed-C 5 µm; THF; UV detection at 280 nm)

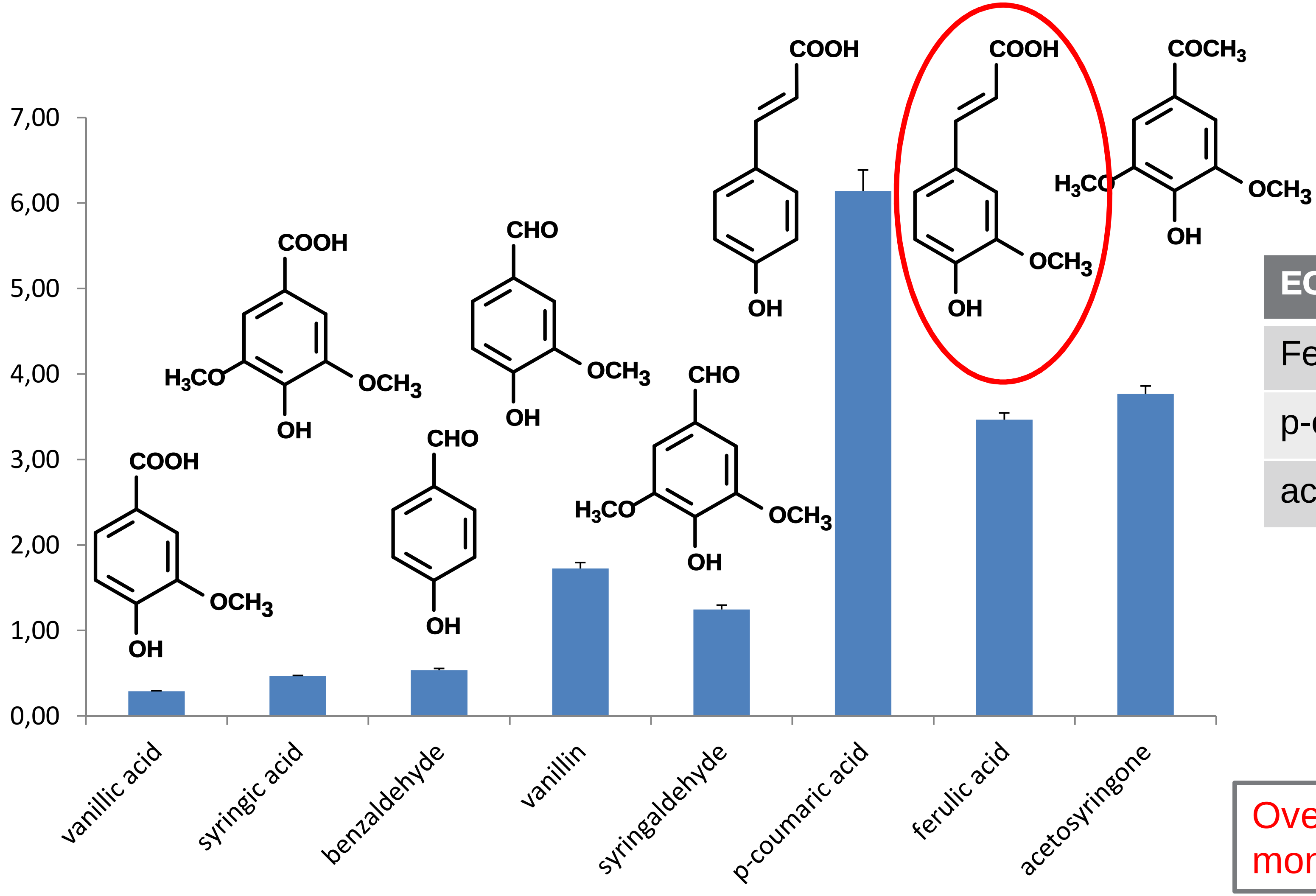
Total free phenolic monomers (mg g^{-1})	
Sarkanda:	0.88 ± 0.03
Protobind:	17.6 ± 0.7
Protobind extracted:	4.2 ± 0.3

by CH_2Cl_2/AcOEt 50/50 v/v

EC50 (g L^{-1} ; dioxane/water 90/10 v/v)	
Sarkanda:	1.04 ± 0.05
Protobind:	0.36 ± 0.01
Protobind extracted:	0.37 ± 0.0



Composition of the Protobind monomer fraction



(50/50 CH_2Cl_2/ethyl acetate extraction; GC-MS)

- ✓ **19% of the fraction**
- ✓ **300-400 lower EC50**

EC50 (g. L^{-1})	
Ferulic acid (FA)	0.05
p-coumaric acid (pCA)	13.5
acetosyringone	19 *

* *Bortolomeazzi et al. 2007*

Over 95% of the RS capacity of the monomer fraction expected from FA

Role in the radical scavenging properties of films: model cellulose-lignin films

Film preparation *(Hambardzumyan et al. 2015)*

- 5/1 cellulose nanocristals/lignin wt. ratio
- Solvent: dioxane/water 0.45/0.55 v/v
- Fenton's reagent: 1 mM $FeSO_4$, 0.1 mM H_2O_2
- pH 3

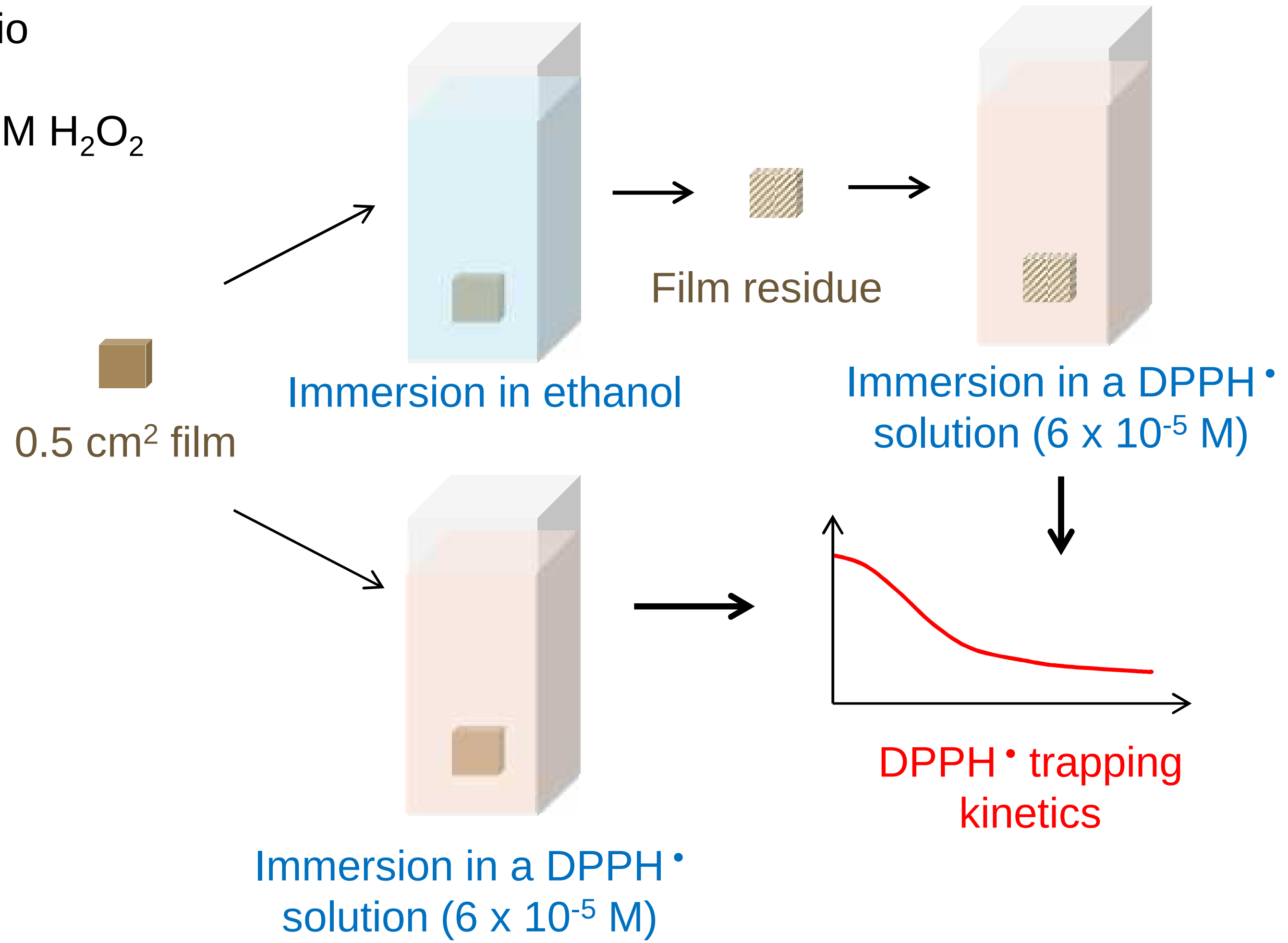
4 h stirring at room T° C

casting onto a PTFE plate

drying (N_2, room T° C, overnight)

Dry NCC-L film (30 ï 5 µm)

DPPH$^\bullet$ test in ethanol *(from Domenek et al. 2013)*



Contribution of the ethanol soluble fraction to the film activity

μmol DPPH$^{\bullet}$ trapped per mg^{-1} film

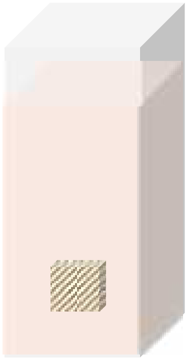
NCC-Protobind:	18.9 ± 1.1
NCC-Protobind extracted:	3.7 ± 0.1

: 5

Total free phenolic monomers (mg g^{-1})

Protobind:	17.6 ± 0.7
Protobind extracted:	4.2 ± 0.3

: 4



Percentage of the activity after immersion

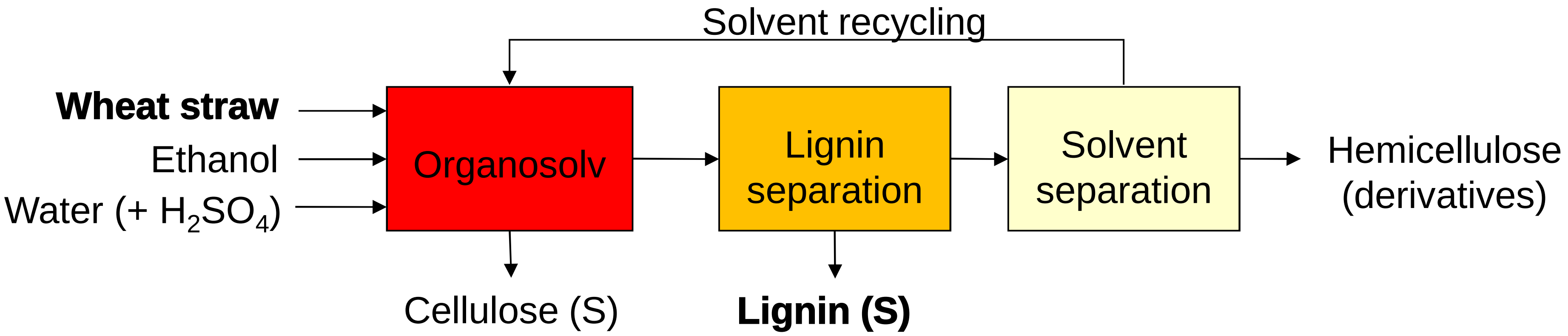
NCC-Protobind:	23%
NCC-Protobind extracted:	52%

- ✓ Protobind extractives determine the film antioxidant capacity in ethanol
- ✓ Ethanol-soluble dimers and oligomers might contribute to the activity in addition to monomers (FA)

Role of ethanol soluble oligomers

Use of three wheat straw lignins samples from different aqueous ethanol organosolv pre-treatment processes:

- OS-A: autocatalytic organosolv (210C)*.
- OS-B: autocatalytic organosolv (200C) following hemicellulose pre-hydrolysis**.
- OS-C: acid-catalysed organosolv at low temperature (140C)***.

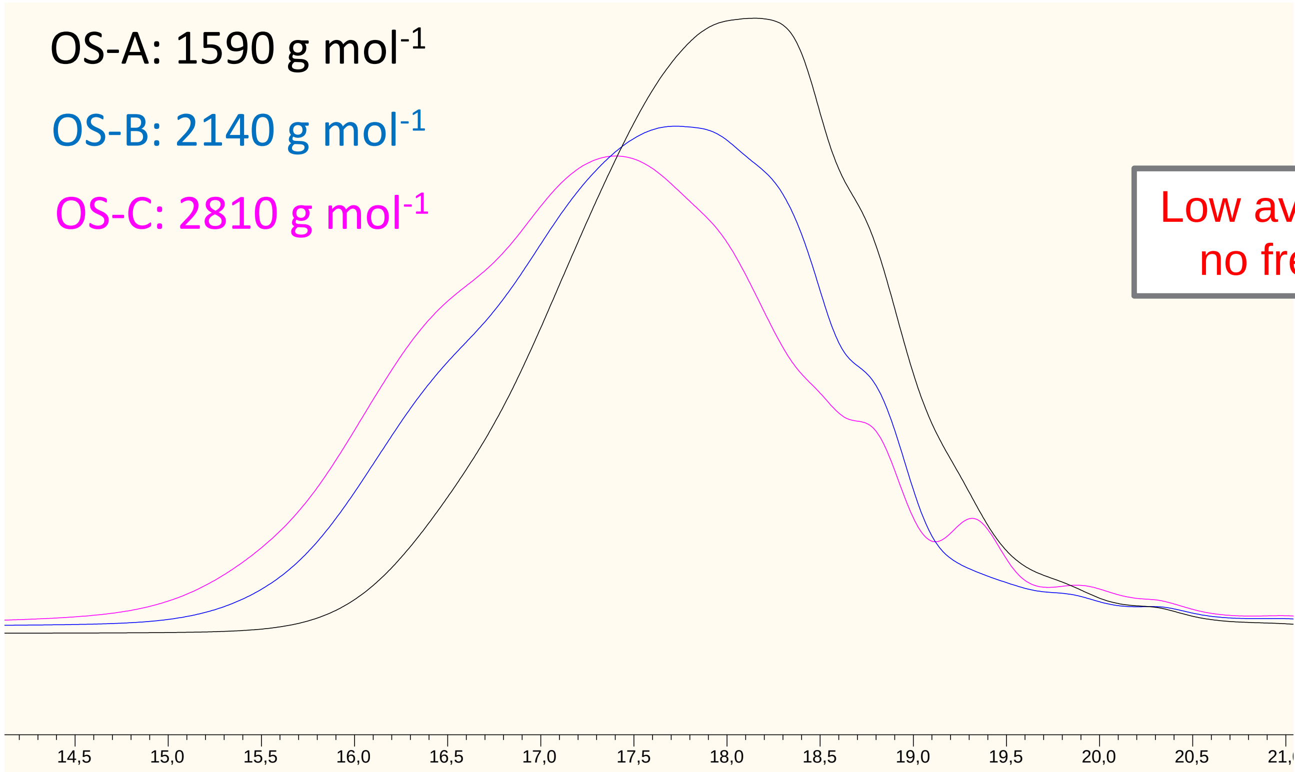


* Huijgen et al. (2012) Bioresour. Technol. 114, 389−398.
** Wildschut et al. (2013) Bioresour. Technol. 135, 58−66.
*** Smit et al. (2014) Patent WO2014126471 A1.

Different depolymerisation degrees reflecting the process severity

Size-exclusion chromatography of the acetylated lignins; 280 nm UV detection

increasing average molar mass (Mw)



Low average molar masses but no free phenolic monomers

Recovery of the ethanol soluble fraction

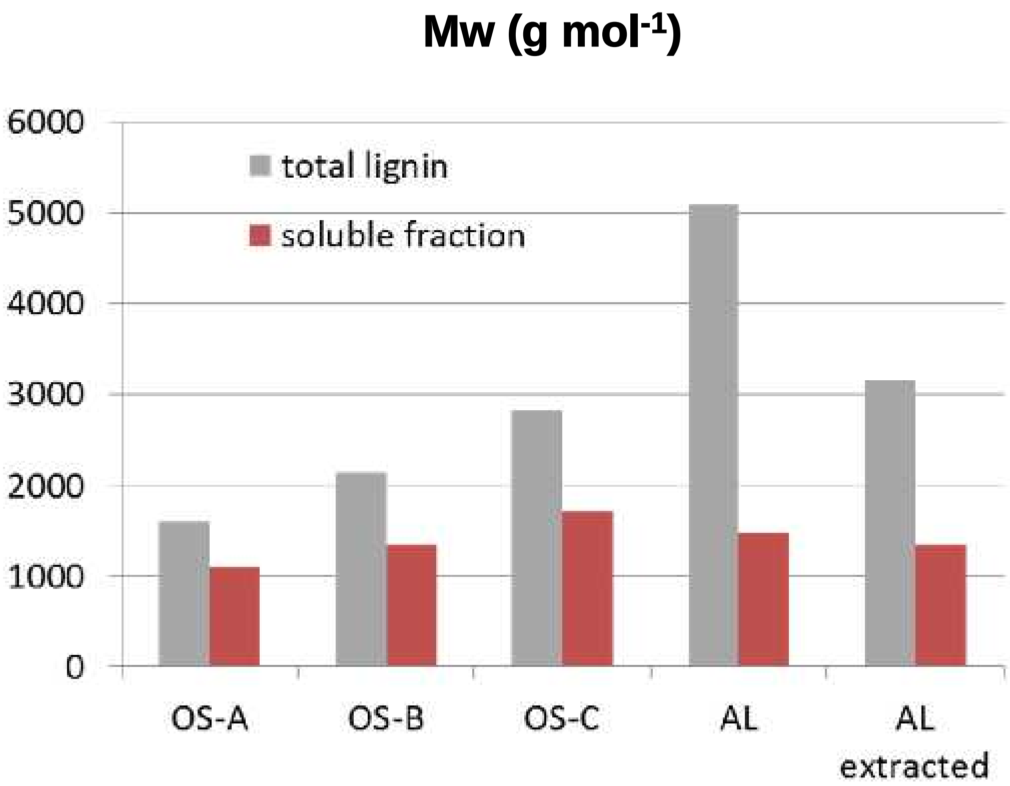
Lignin dispersion in absolute ethanol ; 30 s vortex ; centrifugation 10000 g, 10 min, 20 ° C

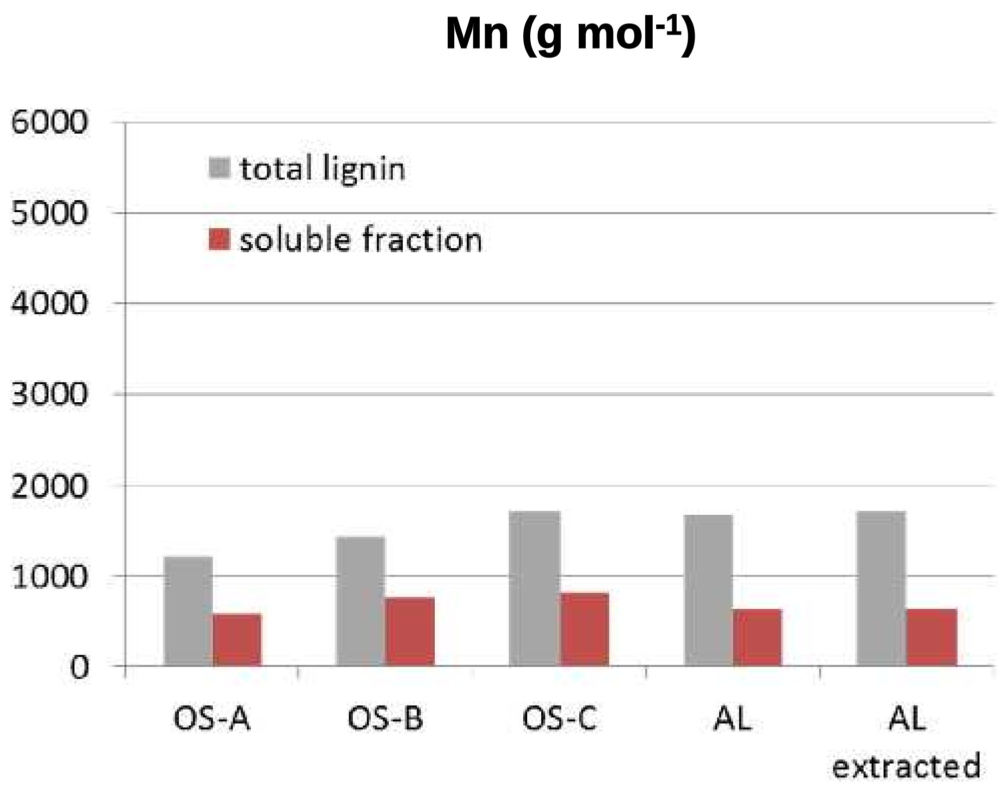
Yields (%): OS-A 67.3±2.6 > OS-B 64.7±2.6 > OS-C 48.0±0.2

Protobind 49.5 ±0.2 > Protobind extracted 40.0 ±0.2

Sample	Total lignin	Ethanol-soluble fraction
	Phenolic OH / aliphatic OH (mol ratio determined by ^{31}P NMR)	
OS-A	2.2	2.0
OS-B	1.4	1.7
OS-C	1.0	1.1
Protobind	1.1	2.0
Protobind extracted	1.3	2.0

Higher proportion of phenolic OH for the alkali lignins but no change for the organosolv ones

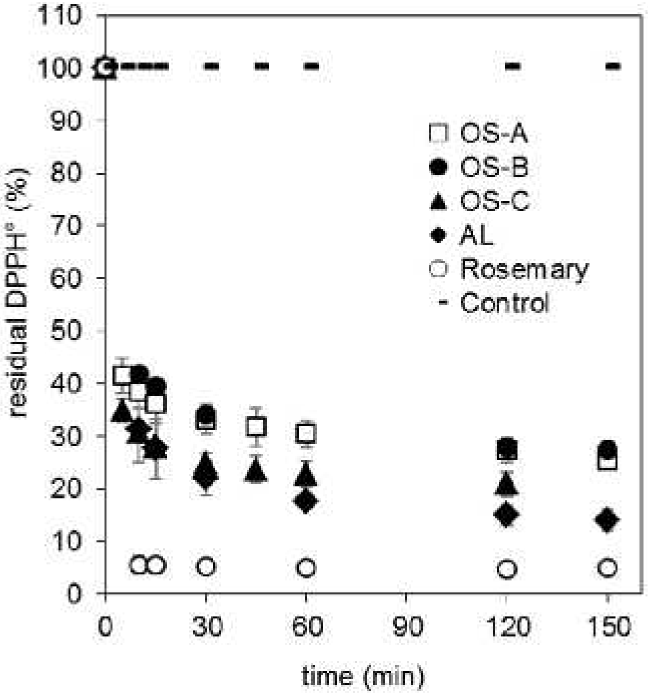




- ✓ Standardisation of the molar mass distribution
- ✓ Selective extraction of oligomers (Mw < DP10)

Antioxidant activity of the ethanolic extracts *(Aguié Béghin et al. 2011)*

Kinetics of DPPH$^{\bullet}$ scavenging in ethanol

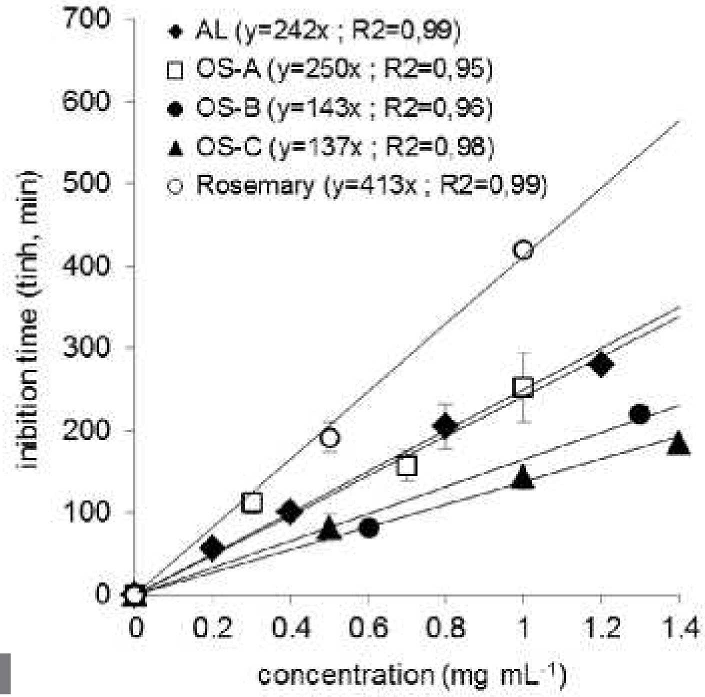


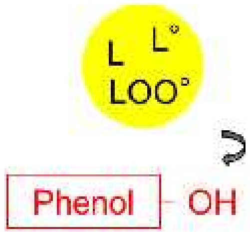
control

lignin extracts

rosemary extract

Inhibition of the AAPH-induced linoleic acid oxidation





	OS-A	OS-B	OS-C	AL
% DPPH trapped by the extract	74.6 ± 4.5	72.7 ± 4.7	79.7 ± 7.6	86.0 ± 11.8
lipid AP (min g^{-1} L)	250	166	154	230

Similar activities with higher radical scavenging of AL extract consistant with higher phenolic OH proportion

Conclusions

- Phenolic oligomers can be selectively extracted by ethanol from alkali and organosolv lignins with a 48-67 % yield

- The lignin ethanolic extracts positively compete with a commercial rosemary extract, the best properties being obtained with the lignin extracts showing the highest phenolic OH proportion

- The non-soluble lignin fraction dispersed in a polymer matrix confer radical scavenging properties to the film while not migrating in the medium

➡ Possibility to produce standardized lignin antioxidant samples for cosmetics and food applications while using the non-soluble residue in plastic materials

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