# 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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## ABSTRACT

The radical scavenging capacity of lignins offers possibility to use them as bio-based antioxidant for high-value applications. Biorefinery grass lignins recovered from an alkaline industrial process and from different ethanol organosolv pretreatments were implemented either in solution or in multiphasic systems. The multiphasic systems consisted of fatty acid aqueous dispersions, as model of food or cosmetic emulsion, and of biodegradable films immersed in ethanol, as model of packaging material in contact with a hydrophobic phase. The lignins exhibited similar radical scavenging properties according to the standard radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH°) test carried out in aqueous dioxane but differences appeared within the multiphasic systems. These differences reflected the proportion of oligomers and monomers likely to diffuse within the system and trap radicals at interfaces. This study shows that ethanol extracts from biorefinery grass lignins could compete with commercial antioxidant additives. Moreover, self-assembling polysaccharide-lignin films could be engineered as active hydrogels for controlled release of antioxidant molecules.

***Keywords:*** *alkali lignins; biorefinery; cellulose nanocomposites; food antioxidant; grass lignins, organosolv pretreatment; radical scavenging*

## INTRODUCTION

Lignins are multifunctional by-products of lignocellulose biorefinery that could advantageously substitute oil-based materials. Their radical scavenging capacity permits to use them as bio-based antioxidants for high-value applications in cosmetics, food packaging or biomedical field, where they positively compete with natural and synthetic antioxidants [1-3]. The combination of the antioxidant properties with antimicrobial, anti-UV and structuring properties give a specific advantage to lignin over other compounds. Though such applications could generate environmental and economic benefits due to increasing market of antioxidants, research is required to provide safe and standardized products by tackling lignin variability.

We focus here on the role of low-molar-mass phenolics on lignin antioxidant properties in two types of matrix: multiphasic matrices representative of food or cosmetic emulsions and polymer matrices representative of packaging and biomedical materials.

## EXPERIMENTAL

### Lignin samples

A commercial alkali lignin (AL) sample (Protobind 1000, mixed *Triticum sp.* and *Saccharum officiarum*) was provided by Green Value SA (Switzerland).

Three organosolv lignins (OS-A, OS-B and OS-C, *Triticum sp.*) were recovered from published ethanolic processes in conditions summarized in table 1 [4-6].

**Table 1. Conditions of the ethanol pretreatment for the production of organosolv lignins**

| Sample | Autoclave volume | Reaction conditions |
|---|---|---|
| OS-A | 20 L | no prehydrolysis<br>cooking: 210°C, 90 min, 50% w/w aqueous ethanol, no catalyst |
| OS-B | 2 L | pre-hydrolysis: 175°C, 30 min, 20 mM $H_2SO_4$<br>cooking: 200°C, 60 min 60% w/w aqueous ethanol |
| OS-C | 2 L | no prehydrolysis<br>cooking: 140°C, 120 min, 60% w/w aqueous ethanol, 60 mM $H_2SO_4$ |

Lignin extracts were recovered from each sample by suspending lignin in absolute ethanol or dioxane/water 90/10 v/v (lignin concentration 0.25-5 mg $mL^{-1}$), homogenization of the dispersion using a vortex and centrifugation at 10000 g for 10 min at 20°C.

The lignin samples were characterized as previously detailed [7].

### Preparation of the films

Cellulose-lignin films were prepared by casting from mixtures of cellulose nanocrystals (CN) and lignin in dioxane/water (0.45/0.55 v/v) with a final cellulose/lignin mass ratio of 5, with the addition of Fenton's reagent (1 mM $FeSO_4$, 0.1 mM $H_2O_2$) [8].

### Antioxidant tests

*Isolated lignins.* The radical scavenging capacity of lignins was assessed by the DPPH° test [9]. 77 µL of lignin aqueous dioxane extract was added to 3 mL of a 6 x $10^{-5}$ M DPPH° solution in dioxane/water 90/10 v/v. The absorbance at 515 nm was monitored and the $EC_{50}$ was determined as the concentration of extract needed to reduce 50% of the initial DPPH° at the plateau. The same test was performed in ethanol with lignin ethanol extracts from 5 mg $mL^{-1}$ lignin suspension, to compare the % DPPH reduced at the plateau.

*Lipid dispersions*. The capacity of lignins to protect free linoleic acid from oxidation was determined by monitoring at 234 nm the production of conjugated diene hydroperoxides generated by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) [10]. The antioxidant power (AP) was calculated as the slope of the curve representing the inhibition time of oxidation vs the concentration of antioxidant.

*Films*. The radical scavenging activity of the films was assessed by immersing a film sample (0.5 $cm^2$; 1.5 mg) into a 6 x $10^{-5}$ M DPPH° ethanolic solution and monitoring the absorbance at 515 nm [11]. The amount of DPPH° reduced at 210 min was calculated and the contribution of migrating substances determined by immersing the film in pure ethanol for 210 min then testing the reactivity of the supernatant toward DPPH°.

## RESULTS AND DISCUSSION

**Table 2. Lignin structural characteristics and antioxidant properties**

| | OS-A | OS-B | OS-C | AL |
|---|---|---|---|---|
| free phenolic monomers (wt%) | 0.25 ± 0.01 | 0.25 ± 0.04 | 0.38 ± 0.00 | 1.9 ± 0.1 |
| ethanol solubility (wt%) | 67.3 ± 2.6 | 64.7 ± 2.6 | 48.0 ± 0.2 | 49.5 ± 0.2 |
| average molar masses (g $mol^{-1}$)[e] and phenolic/aliphatic molar ratio[f] | | | | |
| total fraction | | | | |
| Mw<br>Mn<br>pheOH/aliphOH | 1590<br>1215<br>2.2 | 2140<br>1440<br>1.4 | 2810<br>1710<br>1.0 | 5090<br>1670<br>1.1 |
| ethanol soluble fraction | | | | |
| Mw<br>Mn<br>pheOH/aliphOH | 1100<br>590<br>2.0 | 1340<br>753<br>1.7 | 1710<br>821<br>1.1 | 1480<br>625<br>2.0 |
| antioxidant properties | | | | |
| % DPPH trapped by the ethanolic 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 |
| DPPH° trapped by the film (µmol $mg^{-1}$ film) | 40.8 ± 5.0 | 30.6 ± 2.1 | 16.6 ± 0.3 | 18.9 ± 1.1 |
| contribution of extractives to the film activity (%) | 76 | 74 | 56 | 77 |

**Benefit of lignin ethanolic extraction**

Ethanol was used as possible green biobased solvent to recover lignin extracts suitable for food and cosmetic purposes. The lignin ethanol-soluble fractions account for 48 to 67% of the selected lignins and ethanol led to the selective extraction of oligomers (Mw < 2000 g $mol^{-1}$) and reduction of the variability between the samples. All the ethanolic extracts exhibit DPPH° radical scavenging properties and prevent lipid oxidation with activities similar to a commercial rosemary extract.

**Advantage of highly depolymerized organosolv lignins**

The three OS lignins showed the same radical scavenging capacity in solution ($EC_{50}$ = 0.33 ± 0.04 g of lignin per L). However, better lipid protection by the ethanol extracts was obtained with OS-A and this sample led to the best performances in the cellulose film. This sample was typified by lower average molar masses and a higher proportion of phenolic OH (Table 2), diagnostic of a more depolymerizing treatment. These characteristics are known beneficial to antioxidant properties [11]. We show here that this benefit is exacerbated in multiphasic systems where diffusion of the phenolic molecules is required.

**Role of low-molar-mass phenolics in lignin antioxidant films**

Immersion tests showed that the majority of the film radical scavenging activity was due to lignin ethanol extractable compounds likely to react with DPPH° in the liquid phase. Accordingly, the activity of the films was found correlated to lignin solubility in ethanol.

However, all the films kept a residual activity after immersion, as observed for PLA – AL films [12]. This activity can be due to insoluble lignin fragments and to low-molar-mass compounds grafted through oxidation by Fenton's reagent during the film preparation.

## CONCLUSIONS

1. Ethanol treatment of lignins allows selective extraction of oligomers and recovery of standardized antioxidant extracts competitive with commercial rosemary extracts.

2. Highly depolymerized organosolv lignins are good candidate for usages as antioxidant in food or cosmetic emulsions or polymer films: they combine high solubility in ethanol and high radical scavenging capacity in multiphasic systems.

3. Antioxidant lignin-based materials can be designed either to release antioxidant molecules from hydrogels in biomedical applications or to protect bioproducts by contact without migration of active molecules.

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