CHEMICAL ENGINEERING TRANSACTIONS Volume 24, 2011
Editor Sauro Pierucci
 ISBN 978-88-95608-15-0 ISSN 1974-9791
DOI: 10.3303/CET1124155

Lignin as Natural Radical Scavenger. Study of the Antioxidant Capacity of Apple Tree Pruning Lignin Obtained by Different Methods

Araceli García[a]*, Danila Amendola[b], María González[a], Giorgia Spigno[b], Jalel Labidi[a]

[a] Department of Chemical and Environmental Engineering, University of the Basque Country
Plaza Europa, 1 – 20018 Donostia – San Sebastián, Spain. * araceli.garcia@ehu.es
[b] Institute of Oenology and Food Engineering, Universitá Cattolica Sacro Cuore
Via Emilia Parmense, 84 – 29100 Piacenza, Italy

In the present work, the antioxidant activity of lignins obtained from apple tree pruning was studied. Different procedures were used (autohydrolysis, organosolv and soda processes) and several operation conditions, techniques and methodologies were applied (ultrafiltration and differential precipitation). The obtained lignin samples were characterized by ATR-IR and TGA techniques, and the total phenolic content (Folin-Ciocalteau method) was determined. The antioxidant activity results (evaluated by ABTS assay) showed that: (1) the lignin obtaining process highly affects this property and (2) that the antiradical power of lignin was similar to that observed for two powerful natural antioxidants, gallic acid and catechin, and a commercial one, Trolox®.

1. Introduction

Lignocellulosic material can be used as renewable source of products and chemicals, and their proper exploitation represents an alternative to crude derivatives (García et al., 2010). The biorefinery processes fractionate the lignocellulosic material into its main components: cellulose, hemicelluloses and lignin. Due to its aromatic structure, lignin can be used as source of several products (resins, surfactants …). The antioxidant capacity of lignin has been widely studied and its application in fields as medical, pharmaceutical (anticarcinogenic agent) or polymeric applications (thermal behavior enhancement) has been proved. However, this property depends on the used lignocellulosic material, the extraction methods, and the secondary treatments applied. The aim of the present study is to evaluate the process and treatments that could enhance the antioxidant activity of lignin.

2. Materials and Methods

2.1 Lignin samples obtaining procedures

Different lignin samples were extracted from apple tree pruning residues (w/w composition: moisture 9.06 %, inorganic 9.28 %, lignin 17.00 %, cellulose 30.60 % and hemicelluloses 32.24 %) by several methods and different operation conditions (see

Please cite this article as: Garcia A., Amendola D., González M., Spigno G. and Labidi J., 2011, Lignin as natural radical scavenger. study of the antioxidant capacity apple tree pruning lignin obtained by different methods, Chemical Engineering Transactions, 24, 925-930
DOI: 10.3303/CET1124155

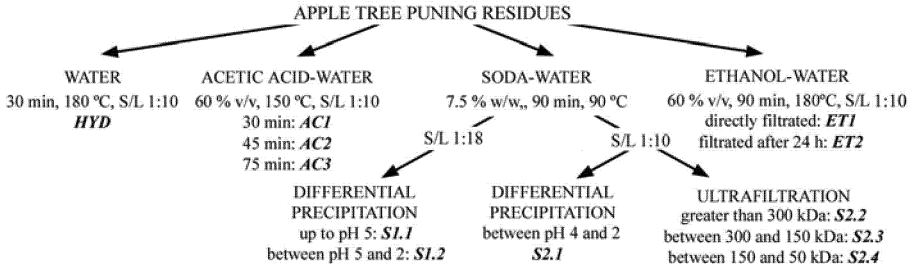

Figure 1: Operation conditions and treatments employed for different lignin samples obtained in this study.

Figure 1). Autohydrolysis (after concentration) and organosolv liquid fractions were treated with two volumes of acidified water (pH 2) for lignin precipitation, which was recovered by filtration (ET2 sample was maintained for 24 h before lignin isolation). Soda liquid fractions were submitted to differential precipitation with 72 % v/v sulfuric acid (S1.2 at pH 5, S1.2 from pH 5 to 2 and S2.1 from pH 4 to 2) or to an ultrafiltration process using 300, 150 and 50 kDa ceramic membranes) and then lignin was precipitated decreasing the pH up to 2 with 72 % v/v sulfuric acid (García et al., 2010).

2.2 Physicochemical analyses of the obtained lignin samples

The chemical structure of the lignin samples was evaluated by ATR-IR spectroscopy and the thermal stability of the lignin samples was studied by Thermogravimetric Analysis (TGA). The analyses were carried out according to the methodologies described in previous works (García et al., 2010).

2.3 Chemical characterization and antioxidant capacity determination

Moisture and ash contents were thermogravimetrically quantified by TGA analysis (from 35 to 800 ºC) in an air oxidizing atmosphere Lignin samples were characterized according to the procedures described by El Mansouri and Salvadó (2006) determining: acid insoluble lignin (AIL), acid soluble lignin (ASL) and glucose, xylose and arabinose contents (Glu, Xyl and Ara respectively).

The total polyphenolic content in lignin samples, catechin and Trolox® was determined by the Folin-Ciocalteau spectrophotometric method (Amendola et al., 2010). Dissolutions of 2 ± 0.5 g/L of lignin in dimethyl sulfoxide (DMSO) were prepared. The total polyphenolic content was determined as mg equivalents of gallic acid per liter of dissolution (GAE) as follows

$$GAE\left(mg/L\right) = mg_{GAE} / L_{lignin\ \ sample\ \ solution} \quad (1)$$

For antioxidant capacity determination different dissolutions of gallic acid, catechin, Trolox® and obtained lignins in DMSO were prepared and analyzed by the ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) assay (Amendola et al., 2010) The antioxidant power (AOP) was determined as:

$$AOP\ (\%) = (A_{blank} - A_{sample}) \cdot 100 / A_{ABTS} \quad (2)$$

In order to understand the effect on the antiradical activity of the composition and the structure of lignin, some specific parameters were defined. The specific antioxidant power per μg of lignin sample (AOP_S) was determined according to the tested lignin concentration (c_{sample}), using the following equation:

$$AOP_S\ (\%/\mu g_{sample}) = AOP / c_{sample} \quad (3)$$

The specific antioxidant power related to the total phenolic content, expressed as AOP per μg equivalents of gallic acid (AOP_G), and AOP per μg of total lignin (AOP_L), were also considered for results interpretation.

$$AOP_G\ (\%/\mu g_{GAE}) = AOP / GAE \quad (4)$$

$$AOP_L\ (\%/\mu g_{lignin}) = AOP / (ASL + AIL) \quad (5)$$

3. Results and Discussion

3.1 Effect of obtaining process on the composition and properties of lignin

Table 1 shows the composition (% w/w) of the obtained lignins using the procedure presented in Figure 1. Autohydrolysis and organosolv samples contained high values of insoluble lignin (up to 87 %). On the other hand alkaline lignins presented high both inorganic (between 15 and 55 %) and hemicellulosic (up to 25 %). In acetic acid extracted samples (AC1, AC2 and AC3) longer reaction times resulted in a decrease of monosaccharides and soluble lignin and in an increase of insoluble lignin. The application of ultrafiltration and differential precipitation also improve the samples purity, because as the membrane cut-off was smaller the sugars content in lignin became lower. Similarly, a previous precipitation (up to pH 5 or 4) eliminates more lignin-hemicelluloses complexes and consequently the precipitated lignins (S1.2 and S2.1) resulted less contaminated.

The thermal stability of the samples, evaluated by the maximum degradation temperature (TG_{max} in Table 1), could be also related to the extraction process. Water and organic solvent allowed obtaining more thermal stable lignins, with TG_{max} between 336.6 and 353.4 ºC. The lower TG_{max} observed in alkaline samples can be related to the hemicellulosic content in the samples that degrade at lower temperatures than the lignin.

Figure 2 shows the ATR-IR spectra of the analyzed lignins. The vibration bands of typical functional groups in the lignin structure were found: aromatic and aliphatic -OH (3400 cm^{-1}), aliphatic and aromatic -CH_2 and -CH_3 (2930, 2850 and 1455 cm^{-1}), non-conjugated carbonyl groups (1735 cm^{-1}), aromatic skeleton (1600, 1510 and 1425 cm^{-1}), syringyl (1330, 1115 and 825 cm^{-1}) and guaiacyl type units (1215, 1025 and 855 cm^{-1}). It can be observed that in autohydrolysis and organosolv lignins (HYD, AC1, AC2, AC3, ET1 and ET2) the intensity of some bands (due to aromatic skeleton, guaiacyl and

Table 1: Chemical composition (% w/w), temperature of maximum degradation (TG_{max}), total phenolic content (GAE mg/L), antioxidant power (AOP %) and specific antioxidant powers (%/µg) of the lignin samples analyzed in the present study.

Sample	H	Ash	IL	SL	Glu	Xyl	Ara	TG_{max} (ºC)	GAE	AOP	AOP_S	AOP_G	AOP_L
HYD	3.45	4.90	87.37	2.28	2.47	2.32	1.00	345.8	751.18	89.73	2.28	5.97	2.44
AC1	7.46	2.64	62.09	1.28	2.30	1.41	n/d	336.6	569.70	97.61	2.35	8.57	3.99
AC2	6.92	4.65	71.64	1.12	2.04	1.48	n/d	347.8	590.91	93.19	2.30	7.88	3.40
AC3	2.91	2.97	82.58	0.96	0.80	1.20	n/d	352.1	683.16	97.97	2.44	7.17	3.15
ET1	4.69	2.72	66.00	1.05	2.63	1.67	0.44	350.6	653.87	89.33	2.20	6.83	3.29
ET2	3.34	7.07	64.50	0.92	2.74	1.80	0.46	353.4	610.10	82.45	2.05	6.76	3.06
S1.1	4.15	45.28	51.09	1.67	3.09	18.70	1.34	290.0	87.96	23.37	0.56	13.29	9.92
S1.2	4.93	40.89	41.61	1.45	2.19	17.64	1.58	243.8	219.53	41.68	1.01	9.49	6.17
S2.1	0.47	55.02	20.33	1.77	3.20	8.79	1.18	209.8	131.00	26.69	0.79	10.19	3.56
S2.2	1.44	47.11	21.82	1.75	2.24	20.67	2.55	232.4	65.91	12.72	0.39	9.65	1.64
S2.3	2.25	37.50	25.48	2.21	2.27	20.90	1.04	252.6	169.94	23.27	0.47	6.85	1.65
S2.4	2.09	14.88	56.48	2.60	1.30	15.62	1.11	256.6	227.77	37.48	1.25	8.23	2.06

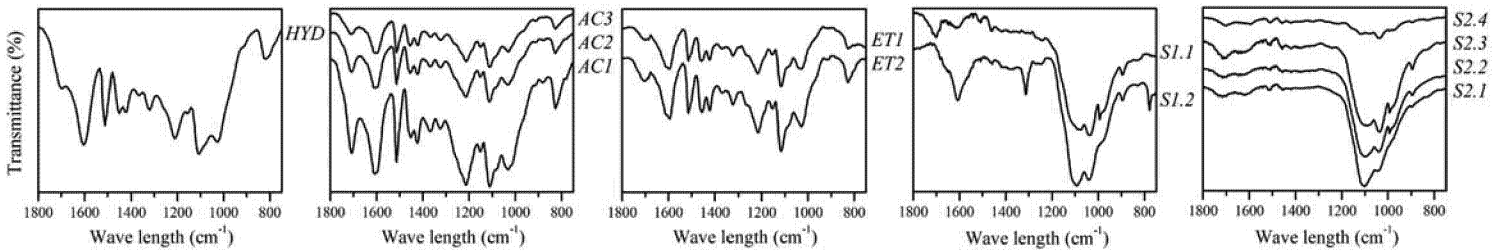

Figure 2: ATR-IR detailed spectra (1800 – 750 cm^{-1}) of the analyzed lignin samples.

syringyl units) are higher than those detected in alkaline samples. This could be interpreted as a major oxidation or degradation of the alkaline lignin structure. The typical broad bands associated to hemicelluloses (at 1160 and 1035 cm^{-1}) are clearly present in soda lignins (S1.1, S1.2, S2.1, S2.2, S2.3 and S2.4). This confirmed the previous results, that higher hemicellulosic impurities were found in alkaline samples.
The phenolic content (GAE in Table 1) resulted to be lower for alkaline lignins. Taking into account the composition results this could be explained by a minor content of lignin in alkaline samples which contained high amounts of ash and sugars, but also it could be due to the severity of the alkaline treatment (oxidation of phenolic groups).

3.2 Antiradical behavior of lignin

The AOP results for the analyzed lignins are shown in Table 1. The antioxidant capacity of the lignin resulted greatly related to the phenolic content in the sample and consequently to the composition, structure and source of the analyzed lignin. In fact, considering the AOP_S, autohydrolysis and organosolv samples resulted between 2 and 7 times more powerful than the alkaline ones. AOP_G indicates the quality of the phenolic compounds presented in lignin samples. Based on this parameter, alkaline samples

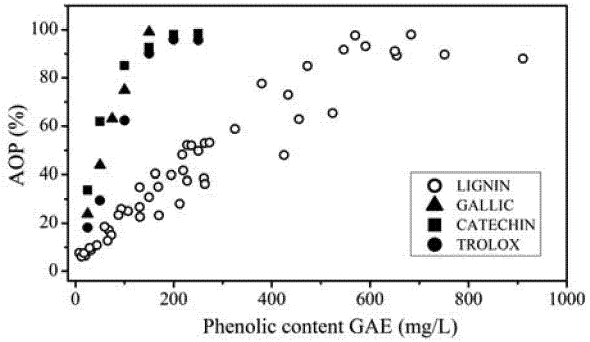

Figure 3: Variation of the antioxidant power (% of reduction) with the phenolic content (mg/L GAE) in analyzed lignin samples and natural/commercial antioxidants

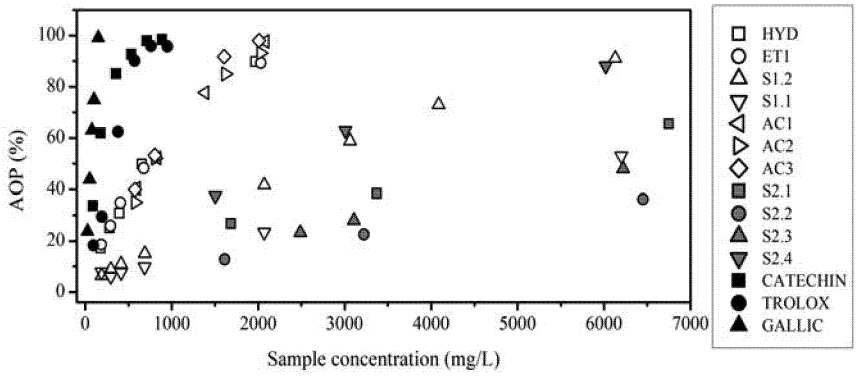

Figure 4: Variation of the antioxidant power (% of reduction) with the amount of analyzed sample (mg/L).

presented less phenolic content but their structure turned out to be more able to reduce the radicals. This could be explained by the action of soda during fractionation process that promoted phenolic compounds oxidation to other aromatic structures that result more adequate for radicals scavenging. Moreover, the AOP_L values (which represent the AOP per μg acid soluble and insoluble lignin fractions) could indicate the influence of lignin purity and structure on its antioxidant capacity. However, this parameter needs to be considered together with the GAE content referred to lignin mass (not shown). Figure 3 shows the antiradical behavior of analyzed lignins and natural and commercial antioxidants (gallic acid, catechin and Trolox®) at different phenolic concentration. It can be observed that lignin polyphenols can reach high antioxidant power (with an apparent similar behavior for all the analyzed samples), but higher contents are necessary to obtain the same activity than gallic, catechin or Trolox® (almost 6 times more). If the AOP related to sample concentration is considered (Figure 4), since lignins do not consist of only polyphenols, lower amounts of gallic acid yield greater antioxidant effect than the same amounts of lignin. Thus the following effectiveness order can be found: gallic>catechin>Trolox®>lignin. For the analyzed samples this order was affected by the lignin source, with autohydrolysis and organosolv lignins providing higher AOP than alkaline ones, and the use of subsequent treatments enhancing the antiradical activity of alkaline samples (S1.2 and S2.4).

4. Conclusions

The obtained results confirmed that the obtaining procedure highly affect the structure, properties and purity of lignin and consequently its antiradical activity. Mild extraction conditions (water and organic solvent) improved sample AOP since they gave high phenolic content lignin and low impurities, while alkali enhanced the capacity of lignin aromatic structure to act as radical scavenger. Alternative treatments (ultrafiltration and differential precipitation) can be used for improving AOP. Lignin was proved to attain similar antiradical activity to those of some powerful and well-known antioxidants.

5. Acknowledgements

The authors would like to thank the Department of Agriculture, Fishing and Food of the Basque Government, the Spanish Ministry of Science and Innovation and the Italian Ministry of Instruction, University and Research (IT2009-0054/ IT09ACBH29) and the Università Cattolica di Piacenza for supporting financially this work.

References

Amendola D., De Faveri D.M., Spigno G., 2010, Grape marc phenolics: Extraction kinetics, quality and stability of extracts, J. Food Eng. 97, 384-392.

El Mansouri N.E., Salvadó J., 2006, Structural characterization of technical lignins for the production of adhesives, Ind. Crops Prod. 24, 8-16.

García A., Toledano A., Andrés M.A., Labidi J., 2010, Study of the antioxidant capacity of *Miscanthus sinensis* lignins, Proc. Biochem. 45, 935-940.