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# Valorisation of lignocellulosic waste materials: Tannins as a source of new products

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## Abstract

Tannins extracted from lignocellulosic industrial wastes, eucalyptus bark and chestnut shell, under different conditions (type and concentration of alkaline chemicals in aqueous solution and temperature), were analysed in order to establish their properties for different potential applications including: their use as phenol substitutes in the formulation of adhesives for wood derivatives and as chrome substitutes in leather tanning, and as a source of alternative antioxidants. Extraction yield was significantly greater for chestnut shell than for eucalyptus bark and the corresponding extracts showed much better properties for the uses proposed. For both materials the highest extraction yield was obtained using 10% NaOH and the lowest using water, however, water extracts showed the best properties. Extraction at 90ºC with a 2.5% $Na_2SO_3$ aqueous solution has been selected as the optimum as extraction yield increased significantly with respect to the extraction with water and the characteristics of the extracts remained almost constant.

Keywords: eucalyptus bark, chestnut shell, biomass conversion, tannins, adhesives

## 1. Introduction

The increase in environmental awareness and in petroleum cost has raised the interest in the exploitation of renewable resources, such as lignocellulosic materials, for the development of new products and processes with a lower environmental impact. Moreover, the valorisation of wastes from industrial productions constitutes a new challenge of economically sustainable and environmentally friendly processes.

*Eucalyptus globulus* wood, one of the main forest species in Galicia (NW of Spain), is mainly used to produce cellulose pulp and wood panels. In both cases, bark is separated as a waste and used as fuel. On the other hand, chestnut (*Castanea sativa)* consumption in the food industry involves about 10000 t/year to produce derivatives

such as marron-glacé, chestnut purée, etc. In the peeling stage chestnut shell is separated and also used as fuel.

In this work, tannins extracted from both industrial lignocellulosic wastes under different conditions, were analysed in order to establish their properties for different potential applications including: their use as phenol substitutes in the formulation of adhesives for wood derivatives and as chrome substitutes in leather tanning, and as a source of alternative antioxidants. The influence of extraction conditions (type and concentration of alkaline chemicals in aqueous solution and temperature) on extraction yield and Stiasny number, tannin content (hide-power test), total phenols content, FRAP (Ferric reducing/antioxidant power) antioxidant capacity and molecular weight distribution of the extracts was analysed. FTIR spectra of crude materials and the extracts obtained were compared.

## 2. Experimental

*Raw materials*
*Eucalyptus globulus* bark and chestnut (*Castanea sativa*) shell were supplied by a pulp factory and a food factory which produces chestnut derivatives, respectively, both in Galicia (NW of Spain).

Both wastes were firstly conditioned in the following steps: air-dried till equilibrium moisture content, ground in a hammer mill, sieved and the fraction of particle size between 0.1 and 2 mm was selected. Chemical composition of chestnut shell and eucalyptus bark is shown in Table 1.

*Extraction and concentration*
The extraction experiments were carried out in a 2-l Pyrex glass reactor with mechanical stirring and temperature control. Chestnut shell or eucalyptus bark and water were mixed at room temperature, heated and, once the selected temperature was attained, the alkali was added and contact time begun to run. 1 h later the suspension was vacuum filtered, the extract together with the first water washings were concentrated by spray-drying and the solid was dried at room temperature in order to calculate the extraction yield as the percentage weight loss of the starting material.

Solid/liquid ratio and contact time were maintained constant at 1/10 (w/w) for chestnut shell and 1/15 (w/w) for eucalyptus bark, respectively and 1 h. Water and different alkaline chemicals such as sodium hydroxide, sodium sulfite, sodium carbonate (alone or combined) in aqueous solution were used as extraction agents at different concentrations and temperature was 70 or 90ºC.

*Antioxidant capacity*
The antioxidant capacity of the extracts was determined by means of the FRAP (Ferric reducing/ antioxidant power) assay, in which the antioxidants present in the sample reduce the Fe(III)/tripyridyltriazine (TPTZ) complex to the blue ferrous form with an increase in absorbance at 593 nm.

The FRAP assay was done according to Szollosi and Szollosi-Varga (2002): 0.1 ml of an aqueous solution of the extracts were transferred to a test tube and 3.0 ml of freshly prepared FRAP reagent (25 ml acetate buffer, 300 mmol/l, pH=3.6; 2.5 ml 10 mmol TPTZ in 40 mmol/l HCl; 2.5 ml 20 mmol/l $FeCl_3$ .$6H_2O$) were added. The absorbance was recorded after 5 min at 593 nm. The relative activities of samples were calculated from the calibration curve of L-ascorbic acid (0.1-1 mmol/l) and the results were expressed as nmole ascorbic acid equivalent (AAE) per mg of extract (on dried basis) (Maksimovic *et al.,* 2005).

*Total phenols content*

Total phenols (TP) content was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965): to 0.5 ml of an aqueous solution of the extract, 2.5 ml of Folin-Ciocalteu reactive, previously diluted with water (1:10 v/v), and 2 ml of aqueous solution of sodium carbonate (75 g/l) were added. The mixture was kept 5 min at 50ºC and, after cooling, the absorbance at 760 nm was measured. The phenols content was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions (2-40 µg/ml) and expressed as mg gallic acid equivalent (GAE) / mg of extract (on dry basis).

*Tannin content (Hyde-power test)*

100 ml of an aqueous solution of extract previously vacuum filtered through a 0.45 µm filter (to determine the soluble solids of the extract) were added to 6.25 g of hyde power. The suspension was stirred for 20 min, then left 10 min at rest, and finally filtered through a sintered glass filter. 50 ml of filtrate were evaporated to determine non- taninns. Tannins were obtained as the difference between soluble solids and non-tannins. The tannin concentration in the aqueous solution of the extract must be between 3.75 and 4.25 g/L. Otherwise the analysis should be repeated adjusting the extract concentration.

*Stiasny number*

The Stiasny number of the extracts, a measure of their formaldehyde condensable polyphenols content, was determined according to the procedure proposed by Yazaki and Hillis (1980). 0.25 mg aqueous solution of extract were dissolved in 100 mL of distilled water. 2.5 ml of 10 M HCl and 5 mL of 37% formaldehyde were added and the mixture was heated under reflux for 30 min. The suspension was filtered through a sintered glass filter, the precipitate washed with hot water and then dried at 105ºC until constant weight.

*GPC*

GPC analysis was performed with an Agilent Technologies 1100 chromatograph equipped with a diode array detector. The column used was a HP-PL gel 5µm Mixed-D protected with a PL gel 5µm guard column. THF was used as eluent and the conditions used were: flow rate, 1 ml/min; column temperature, 30ºC; injection volume, 20 µl; detection at 270 with a bandwidth of 15 nm. The calibration curve was obtained with polystyrene standards.

The spray-dried extracts were acetylated previous to GPC analysis. Samples (20 mg) were acetylated with pyridine-acetic anhydride (1:1, 4 mL) overnight at room temperature. The mixture was poured into distilled water (40 ml) and the precipitate obtained was recovered by vacuum filtration through a 20 μm nylon filter. The acetylated extracts were dissolved in THF (2-5 mg/ mL) and analysed by GPC.

*FTIR spectra*
FTIR spectra were determined in a Bruker IFS-66v spectrometer. Samples were prepared using the KBr pellet technique (2.5% sample). FTIR spectra were recorded at 4 $cm^{-1}$ resolution and 32 scans were accumulated prior to Fourier transform.

## 3. Results and discussion

Chemical composition of chestnut shell and eucalyptus bark is shown in Table 1. Chestnut shell contains more extracts and lignin but less carbohydrates and ash than eucalyptus bark.

Table 1. Composition of chestnut (*Castanea sativa*) shell and *Eucalyptus globulus* bark

| | **Chestnut shell (% o.d.shell)** | **Eucalyptus bark (% o.d.bark)** |
|---|---|---|
| **Ash** | 0.83 | 4.74 |
| **Extracts:** | | |
| **Cold water** | 3.85 | 2.59 |
| **Hot water** | 26.87 | 5.31 |
| **1% NaOH** | 71.32 | 26.58 |
| **Ethanol+Ethanol-Benzene+Water** | 34.52 | 19.20 |
| **Klason lignin** | 29.15 | 16.73 |
| **Soluble lignin** | 2.83 | 2.48 |
| **Cellulose** | 19.20 | 41.63 |
| **Total sugars** | 33.82 | 62.47 |
| ***Monosaccharides*:** | 32.70 | 60.98 |
| **Glucose** | 19.23 | 42.91 |
| **Galactose** | 2.98 | 2.21 |
| **Xylose** | 6.45 | 12.72 |
| **Arabinose** | 2.52 | 2.26 |
| **Mannose** | 1.52 | 0.88 |

Table 2 shows for both materials the results obtained for extraction yield and Stiasny number, total phenols content and FRAP (Ferric reducing/antioxidant capacity) of the extracts obtained varying the type and concentration of alkaline chemicals in aqueous solution and temperature.

Extraction yield was significantly greater for chestnut shell than for eucalyptus bark. For both materials the highest extraction yield was obtained using 10% NaOH and the lowest using water (49.4 and 8.7% for chestnut shell and 18.9 and 6.8% for eucalyptus bark). Moreover chestnut shell extracts showed, for all the extraction

conditions essayed, remarkably higher Stiasny number, phenol and tannin contents and antioxidant capacity.

Table 2. Extraction yield at different extraction conditions for chestnut shell and eucalyptus bark and characteristics of the corresponding extracts

| | Chestnut shell | | | | Eucalyptus bark | | | |
|---|---|---|---|---|---|---|---|---|
| | **Extraction yield (%)** | **Stiasny number** | **Total phenols (gEAG/ g extract)** | **FRAP (nmol AAE/mg extract)** | **Extraction yield (%)** | **Stiasny number** | **Total phenols (gEAG/ g extract)** | **FRAP (nmol AAE/mg extract)** |
| **Water 70ºC** | 8.71 | 111.4 | 0.4822 | 2486 | - | - | - | - |
| **Water 90ºC** | 12.2 | 105.8 | 0.5577 | 3555 | 6.8 | 37.6 | 0.1809 | 912 |
| **NaOH 2.5%-70ºC** | 27.58 | 87.8 | 0.3531 | 1593 | 9.81 | 23.1 | 0.0543 | 176 |
| **NaOH 2.5%-90ºC** | 34.55 | 85.7 | 0.4184 | 2721 | 10.83 | 23.5 | 0.0877 | 227 |
| **NaOH 10%-70ºC** | 49.38 | 75.2 | 0.2354 | 624 | 16.39 | 14.3 | 0.0363 | 162 |
| **NaOH 10%-90ºC** | - | - | - | - | 18.9 | 16.5 | 0.054 | 164 |
| **$Na_2S_2O_3$ 2.5%-70ºC** | 14.61 | 99.2 | 0.5217 | 3427 | 6.8 | 28.9 | 0.1439 | 725 |
| **$Na_2S_2O_3$ 2.5%-90ºC** | 25.62 | 95.1 | 0.5227 | 3150 | 8.55 | 35.5 | 0.1864 | 914 |
| **$Na_2S_2O_3$ 10%-70ºC** | 28.79 | 77.3 | 0.3228 | 1765 | 6.81 | 9.7 | 0.0915 | 394 |
| **$Na_2S_2O_3$ 10%-90ºC** | - | - | - | - | 10.19 | 15.3 | 0.1248 | 595 |
| **NaOH 2.5% $Na_2S_2O_3$ 2.5%-70ºC** | 35.97 | 88.8 | 0.2975 | 1312 | 7.94 | 8.9 | 0.0715 | 266 |
| **NaOH 2.5% $Na_2S_2O_3$ 2.5%-90ºC** | 44.38 | 88.8 | 0.4244 | 2380 | 12.3 | 12 | 0.0917 | 326 |
| **NaOH 5% $Na_2S_2O_3$ 5%-70ºC** | 45.86 | 78.9 | 0.3213 | 1627 | 13.25 | 5.3 | 0.0502 | 240 |
| **NaOH 5% $Na_2S_2O_3$ 5%-90ºC** | - | - | - | - | 16.19 | 5.2 | 0.0656 | 204 |
| **$Na_2CO_3$ 2.5% $Na_2S_2O_3$ 2.5%-70ºC** | 23.94 | 90 | 0.3973 | 2455 | - | - | - | - |
| **$Na_2CO_3$ 2.5% $Na_2S_2O_3$ 2.5%-90ºC** | - | - | - | - | 10.72 | 14.3 | 0.103 | 352 |
| **$Na_2CO_3$ 5% $Na_2S_2O_3$ 5%-70ºC** | 32.83 | 84.1 | 0.3008 | 1441 | - | - | - | . |
| **$Na_2CO_3$ 5% $Na_2S_2O_3$ 5%-90ºC** | - | - | - | - | 10.7 | 5.1 | 0.0552 | 170 |

The Stiasny number, the total phenols content and the antioxidant capacity decreased when the alkalinity of the solution was increased. With respect to the influence of temperature, an increase from 70 to 90ºC implied, for all the alkaline compounds used, not only an increase in the extraction yield but also an improvement in the extract properties. Although water extracts showed the best properties, 90º C, 2.5% $Na_2SO_3$ extracts joined a higher extraction yield and only slightly worse characteristics of the extracts. For both materials linear relationships were found among Stiasny number, tannin content, total phenols content and antioxidant capacity of the corresponding extracts, as shown in Fig. 2 and 3 for antioxidant capacity and phenols content and for tannin content versus phenol content, respectively.

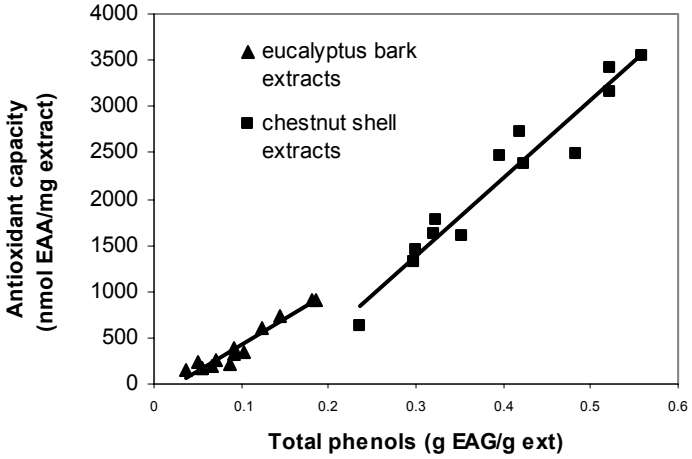


Fig. 2.- Antioxidant capacity versus total phenols for chestnut shell and eucalyptus bark extracts

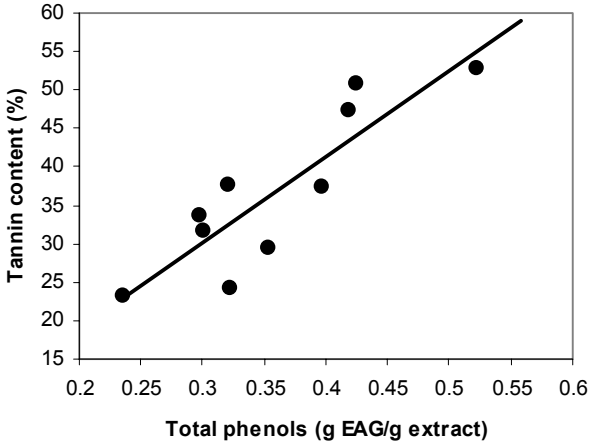


Fig. 3.- Tannin content versus total phenols content for chestnut shell extracts

FTIR spectra of chestnut shell and eucalyptus bark extracts were compared and with those of the corresponding raw materials. From Fig. 4 differences between the 2.5% $Na_2SO_3$ extracts of both materials could be established. Both the 1650-1450 $cm^{-1}$ absorption band, attributed to aromatic ring stretching vibration, and the OH in plane deformation absorption band at 1420-1330 $cm^{-1}$ were stronger for chestnut shell extracts than for eucalyptus bark extracts, indicating than the former contained more aromatic compounds and polyphenols than the latter.

Molecular weight distribution was in most cases bimodal for chestnut shell extracts whereas eucalyptus bark extracts showed multimodal distributions. Average molecular weights of water chestnut shell extracts were the highest among chestnut extracts and significantly higher than those of water eucalyptus bark extracts. Both number and weight average molecular weights of the extracts diminished when increasing the severity of the alkaline extraction for chestnut extracts but increased slightly for eucalyptus extracts.

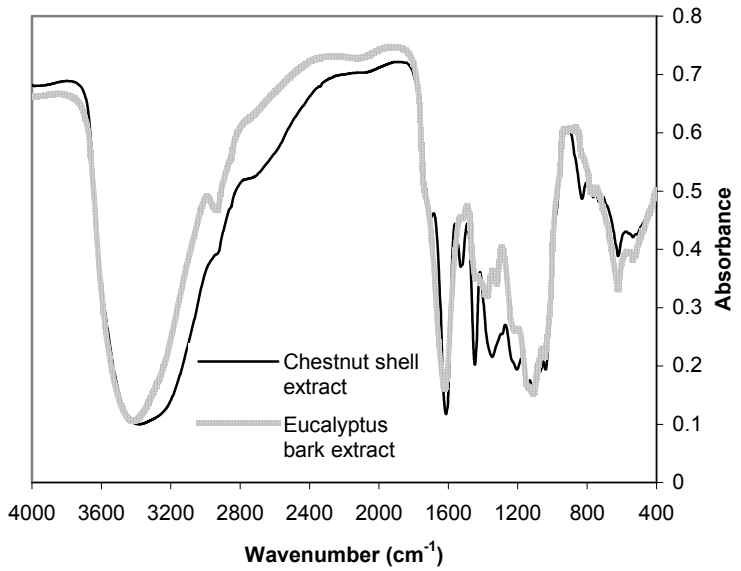


Fig. 4.- FTIR spectra of 2.5% $Na_2SO_3$ extracts of chestnut shell and eucalyptus bark

For both materials, extraction at 90ºC with a 2.5% $Na_2SO_3$ aqueous solution has been selected as extraction yield increased significantly with respect to the extraction with water and the characteristics of the extracts remained almost constant. On the other hand, it can be concluded than chestnut shell tannin extracts, due to their significantly higher polyphenols contents, offer much better properties than eucalyptus bark extracts for all the applications proposed.

## 4. Acknowledgements

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