Study of the antioxidant potential of forestry biomass waste

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Abstract

In the present study, the chemical composition of hazelnut husks, mimosa wood and waste forestry biomass was determined and their potential as sources of antioxidant compounds was evaluated. Extractions with water, ethanol, methanol, 50% ethanol and 50% methanol at 50ºC for 90 min were carried out to analyze the effect of the solvent on extraction yield and on the extracts' total phenol content and antioxidant properties (FRAP, DPPH and ABTS assays). Mimosa wood extracts showed the highest total phenol content (27.86 g GAE/100 g extract) and FRAP (1889 nmol AAE/100 g extract) and the lowest EC_{50} ABTS (0.556 mg/mL) value, while waste forestry biomass showed the lowest EC_{50} DPPH (0.076 mg/mL) value. The highest extraction yields were obtained for hazelnut husks and the lowest for waste forestry biomass. The solvent that led to the best extract properties depended on the material, being 50% ethanol for hazelnut husks, ethanol for mimosa wood and 50% methanol for waste forestry biomass. The extracts obtained under the best conditions selected were analyzed by RP-HPLC-ESI-TOF to identify the phenolics responsible for the antioxidant activity. GPC analysis revealed the predominance of compounds of low and medium molecular weight.
Keywords: hazelnut husks, mimosa wood, waste forestry biomass, phenolic compounds, antioxidant activity, GPC, RP-HPLC-ESI-TOF.

1 Introduction

Antioxidants have been widely used in the food industry to avoid the oxidative deterioration of fats and oils that affect flavour, nutritional quality and safety of

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food products. However, the safety of some synthetic antioxidants, such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) is being questioned [1–3] and investigations have been oriented towards finding new sources of natural antioxidants. At present, the interest is focused on polyphenols, compounds with demonstrated antioxidant capacity and present in many plant materials [4]. Especially, the obtaining of natural antioxidants from biomass waste by-products from food, wood and agricultural industries has been studied [2, 4–7] promoted by the advantage of their low cost and the environmental benefits related with their re-use.

In this work, the potential of three Galician (NW of Spain) forestry waste: hazelnut (*Corylus avellana*) husk, mimosa (*Acacia dealbata*) wood and waste forestry biomass, for obtaining phenolic antioxidants was studied. Hazelnut husk is a residue related to hazelnut processing in the food industry whereas mimosa wood, an invasive tree of the Galician forest, and waste forestry biomass, a mixture of wood, bark, leaves and fruits from *Eucalyptus globulus* and *Pinus pinaster,* are linked to forest cleaning operations. Firstly, the chemical composition of the materials was analysed by determining the main fractions constituent of lignocellulosic materials and their ultimate analysis. Secondly, the effect of the type and concentration of the solvent (water, ethanol or methanol) on extraction yield and on the total phenols content and antioxidant properties of the extracts was studied and the results for the three materials compared. Finally, the extracts with the best antioxidant properties were analyzed by gel permeation chromatography (GPC) to obtain their molecular weight distribution and by RP-HPLC-ESI-TOF to obtain their polyphenolic profile.

2 Materials and methods

2.1 Raw material and chemical composition

Hazelnut husk and mimosa wood were collected in Ourense (Galicia, NW Spain). Waste forestry biomass was supplied by the biomass power plant Allarluz S.A. (Allariz, Spain). The materials were air-dried till equilibrium humidity, ground and the fraction of particle size between 0.1 and 1 mm was selected. The chemical composition of the materials was determined: ash (ASTM D1102-84); water solubility (ASTM D1110-84); 1% NaOH solubility (ASTMD1109-84); acid-insoluble lignin (ASTM D1106-84); acid-soluble lignin by the spectrophotometric method of Maekawa *et al.* [8]; cellulose content, by treatment with nitric acid/acetic acid [9]; total sugar content, by the phenol/sulphuric acid method after hydrolysis of the polysaccharides [10]. The ultimate analysis was performed in an Ultimate Analyzer Thermo Finnigan Flash 1112. All analyses were carried out in triplicate and the results averaged.

2.2 Extraction and concentration

Hazelnut husks, mimosa wood and waste forestry biomass were extracted with water, ethanol, methanol, 50% ethanol and 50% methanol for 90 min at 50ºC.

The extraction experiments were carried out in 1-L Pyrex glass flasks in an orbital shaker at a solid/liquid ratio of 1/10 (w/w) and a shaking speed of 90 rpm. The extract was recovered by vacuum filtration and the extraction yield was calculated as the percentage weight loss of the starting material. The solvent was evaporated in a Büchi R-210 rotavapor except for the aqueous extract that was concentrated by spray-drying. Extractions and all extract analysis were carried out in triplicate and the results averaged.

2.3 Total phenols content

Total phenols content (TPC) was determined by the Folin-Ciocalteu method: 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 a 75 g/L Na_2CO_3 aqueous solution were added. The mixture was kept 5 min at 50 ºC and, after cooling, the absorbance at 760 nm was measured. The phenols content was expressed as g gallic acid equivalent (GAE)/100 g extract (on dried basis).

2.4 Antioxidant activity

2.4.1 Ferric reducing antioxidant power (FRAP)

The FRAP assay was done as follows: 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 (2,4,6-tripyridyl-s-triazine) in 40 mmol/L HCl; 2.5 mL 20 mmol/L $FeCl_3 \cdot 6H_2O$) were added. The absorbance was recorded after 5 min at 593 nm. The relative activities of samples were expressed as nmol ascorbic acid equivalent (AAE) per mg extract (on dried basis).

2.4.2 DPPH radical-scavenging activity

Aqueous solutions of extracts (8-500 μg/mL) were prepared. The extract solution (0.3 mL) was mixed with 2.7 mL of a freshly prepared DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (6.10^{-5} M in 80% methanol). The mixture was shaken vigorously and left to stand for 20 min in the dark at room temperature. Then the absorbance was read at 517 nm. The radical-scavenging activity (RSA) was determined as $\%RSA=100\ (A_0-A_s)/A_0$, where A_s is the absorbance of the extract solution and A_0 is the absorbance of a control solution prepared without extracts. The Trolox equivalent of the extracts (TRE) was calculated and expressed as mmol Trolox equivalent (TRE) per g extract (on dried basis). The EC_{50} value or extract concentration necessary to achieve a 50% radical DPPH inhibition, were obtained by plotting the %RSA as a function of sample concentration.

2.4.3 ABTS radical-scavenging activity

ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) was produced by reacting an ABTS solution (7 mM) with potassium persulfate (2.45 mM) for 16 h in the dark at room temperature. The $ABTS^{\bullet+}$ solution was diluted with water to an absorbance of 0.70 at 734 nm. Aqueous solutions of chestnut bur extracts (20-1000 μg/mL) were prepared. The extract

solution (25 μL) was mixed with the $ABTS^{\bullet+}$ solution (2.5 mL) and after 6 min in the dark at room temperature the absorbance was read at 734 nm. The % RSA of the extract solutions, the extracts TRE and the EC_{50} values were calculated as indicated in the DPPH method.

2.5 Molecular weight distribution and average-molecular-weights by GPC

Acetylated extracts [6] were dissolved in THF (2-5 mg/mL) and analysed by GPC 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 nm with a bandwidth of 15 nm. The calibration curve was obtained with polystyrene standards.

2.6 RP-HPLC-ESI-TOF mass spectrometry

Extracts were evaluated using an Agilent Technologies 1100 HPLC and a Bruker Microtof ESI-TOF instrument. Polyphenols were separated using a Zorbax Eclipse XDB-C18 5 μm (4.6x150 mm) column and a binary gradient of 2% acetic acid for mobile phase A and 0.5% acetic acid in water/acetonitrile (1:1, v/v) for mobile phase B at a flow rate of 1 mL/min. The linear gradient was from 10 to 55% B from 0 to 50 min, from 55 to 100% B from 50 to 60 min and from 100 to 10% B from 60 to 65 min. The mass spectrometry analysis was performed in negative ion mode. The samples were dissolved in MeOH/H_2O (1:1, v/v) to a concentration of 25 mg/ml.

2.7 Statistical analysis

The existence of significant differences among the results for extraction yield, total phenols content and antioxidant properties of the extracts depending on the solvent used was analysed. The one-way analysis of variance (ANOVA) was used, followed by the Tukey's HSD or Dunnett T3 test depending on whether equal variances could be assumed or not. All statistical tests were performed at a 5% significance level using PASW Statistics 18 software.

3 Results and discussion

3.1 Chemical composition

The chemical composition and ultimate analysis of hazelnut husks, mimosa wood and waste forestry biomass are shown in table 1. Hazelnut husks showed the highest ash and extractable compounds contents and the lowest lignin and carbohydrates contents. The cellulose and total sugar contents of mimosa wood and waste forestry biomass were similar, on the contrary, the lignin content of the latter was significantly higher.

Table 1: Chemical composition and ultimate analysis of hazelnut husks, mimosa wood and waste forestry biomass.

Chemical composition	Hazelnut husks	Mimosa wood	Waste forestry biomass
	% dry basis		
Ash	10.03 ± 0.54	0.55 ± 0.015	2.56 ±0.21
Cold water extracts	33.79 ± 0.76	8.78 ± 0.09	20.39 ± 0.48
Hot water extracts	36.17 ± 0.76	11.36 ± 0.09	24.75 ± 0.41
1% NaOH extracts	60.15 ± 0.8	29.49 ± 0.31	46.45 ± 0.30
EtOH-toluene extracts	10.55 ± 0.55	4.63 ± 0.45	12.90 ± 0.18
Cellulose	20.26 ± 0.63	38.51 ± 1.17	36.62 ± 0.91
Total sugars	23.62 ± 0.96	49.82 ± 3.28	50.43 ± 0.77
Acid-insoluble lignin	15.12 ± 0.13	18.24 ± 0.29	26.32 ± 0.16
Acid-soluble lignin	1.85 ± 0.08	2.50 ± 0.09	2.53 ± 0.32
Ultimate analysis			
Carbon	41.6 ± 0.13	44.6 ± 0.45	45.26 ± 0.04
Hydrogen	5.58 ± 0.02	6.19 ± 0.08	5.83 ± 0.33
Oxygen	51.75 ± 0.08	48.84 ± 0.39	48.57 ± 0.42
Nitrogen	0.99 ± 0.11	0.39 ± 0.01	0.32 ± 0.01
Sulphur	0.04 ± 0.06	0.00 ± 0.00	0.03 ± 0.04

3.2 Extraction with solvents

Hazelnut husks, mimosa wood and waste forestry biomass were extracted with water, ethanol, methanol and their 50% aqueous solutions to analyze the influence of the solvent on extraction yield and extract antioxidant properties. The results are shown in table 2.

Comparing the three materials, the highest extraction yields were obtained for hazelnut husks and the lowest for waste forestry biomass, independently of the solvent used. For hazelnut husks and mimosa wood, the recovery of extractable compounds was higher with aqueous ethanol and methanol than with the pure solvents. However, for waste forestry biomass no significant differences were found among the extraction yields obtained with ethanol, methanol and their aqueous solutions, and water led to the lowest value.

3.3 Total phenols content and antioxidant activity

For the three materials tested, the solvent used result to be a significant factor on total phenols content and antioxidant properties of the extracts ($p < 0.05$).

For hazelnut husks the solvent that provided the best extract properties was 50% ethanol. The total phenols content and antioxidant properties of the extracts increased in the following order: water < ethanol < methanol < 50% methanol < 50% ethanol. The results obtained with 50% ethanol and 50% methanol were similar and, except for DPPH Trolox equivalent values, no significant

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Table 2: Extraction yield, total phenols content and antioxidant properties of hazelnut husks, mimosa wood and waste forestry biomass extracts.

	Extraction yield (%)	TPC (g GAE/ 100 g extract)	FRAP (nmol AAE/ mg extract)	DPPH		ABTS	
				mmol TRE/ g extract	EC_{50} (mg/ml)	mmol TRE/ g extract	EC_{50} (mg/ml)
Hazelnut husks							
Water	30.06 ± 0.92^{bc}	5.52 ± 0.44^{a}	537 ± 23^{a}	0.47 ± 0.03^{a}	0.344 ± 0.019^{a}	0.68 ± 0.03^{a}	2.479 ± 0.092^{a}
EtOH	25.15 ± 0.73^{a}	11.27 ± 0.17^{b}	801 ± 18^{b}	0.75 ± 0.01^{b}	0.216 ± 0.003^{d}	1.15 ± 0.04^{c}	1.459 ± 0.053^{b}
MeOH	28.29 ± 0.31^{b}	12.03 ± 0.22^{c}	826 ± 23^{b}	0.83 ± 0.01^{c}	0.197 ± 0.002^{cd}	1.39 ± 0.08^{d}	1.215 ± 0.067^{c}
50% EtOH	32.41 ± 1.02^{c}	13.43 ± 0.20^{d}	961 ± 12^{c}	1.05 ± 0.01^{d}	0.155 ± 0.002^{b}	1.70 ± 0.01^{b}	0.988 ± 0.008^{c}
50% MeOH	31.02 ± 0.06^{bc}	12.29 ± 0.88^{bcd}	$881 \pm 53b^{c}$	0.97 ± 0.01^{e}	0.168 ± 0.001^{bc}	1.54 ± 0.01^{db}	1.092 ± 0.007^{c}
Mimosa wood							
Water	8.85 ± 0.66^{a}	8.91 ± 0.45^{a}	659 ± 17^{a}	0.47 ± 0.01^{a}	0.344 ± 0.007^{a}	1.12 ± 0.09^{a}	1.510 ± 0.115^{a}
EtOH	11.02 ± 0.41^{ab}	27.86 ± 0.72^{b}	1889 ± 17^{b}	1.67 ± 0.01^{c}	0.096 ± 0.001^{b}	3.02 ± 0.08^{b}	0.556 ± 0.014^{b}
MeOH	12.23 ± 1.96^{ab}	25.31 ± 0.49^{c}	1733 ± 18^{c}	1.40 ± 0.04^{b}	0.115 ± 0.004^{c}	2.71 ± 0.04^{c}	0.620 ± 0.008^{c}
50% EtOH	16.33 ± 0.57^{c}	25.69 ± 1.62^{bc}	1488 ± 63^{d}	1.47 ± 0.02^{b}	0.109 ± 0.001^{bc}	2.73 ± 0.05^{c}	0.615 ± 0.010^{c}
50% MeOH	$14.92 \pm 0.38b^{c}$	24.04 ± 0.82^{c}	1408 ± 19^{d}	1.42 ± 0.00^{b}	0.113 ± 0.000^{c}	2.56 ± 0.01^{c}	0.657 ± 0.003^{c}
Waste forestry biomass							
Water	7.36 ± 0.03^{a}	19.27 ± 0.39^{c}	978 ± 19^{b}	1.36 ± 0.01^{b}	0.125 ± 0.001^{c}	2.32 ± 0.12^{b}	0.853 ± 0.042^{bc}
EtOH	10.23 ± 0.07^{b}	13.14 ± 0.56^{a}	579 ± 45^{a}	0.68 ± 0.05^{a}	0.251 ± 0.017^{a}	1.19 ± 0.09^{a}	1.672 ± 0.130^{a}
MeOH	11.88 ± 1.06^{b}	17.50 ± 0.50^{b}	891 ± 11^{c}	$1.17\ 0.03^{b}$	0.145 ± 0.003^{c}	1.95 ± 0.11^{c}	1.017 ± 0.058^{c}
50% EtOH	11.82 ± 0.89^{b}	21.82 ± 0.22^{d}	1198 ± 28^{d}	1.96 ± 0.11^{c}	0.087 ± 0.005^{b}	2.53 ± 0.04^{d}	0.782 ± 0.012^{bc}
50% MeOH	10.73 ± 0.41^{b}	25.70 ± 0.40^{e}	1496 ± 10^{e}	2.23 ± 0.04^{d}	0.076 ± 0.001^{b}	2.96 ± 0.06^{e}	0.668 ± 0.014^{b}

In each column for each material, different letters mean significant differences at $p < 0.05$.

differences were found between them. Comparing the results with those obtained for other hazelnut by-products, the antioxidant properties of hazelnut husks extracts were lower than those of roasted hazelnut skin [5] (FRAP: 4373 nmol AAE/mg; ABTS: 5.42 mmol TRE/g) but higher than those of hazelnut shell [2] (ABTS: 0.117–0.148 mmol TRE/g).

With respect to mimosa wood, the best extract properties were obtained with pure ethanol and the worst with water. Extracts with similar antioxidant properties were obtained with methanol, 50% ethanol and 50% methanol and no significant differences were found for extract total phenols content and radical scavenging capacities. The DPPH antioxidant activity was of the same order as that found for the stem bark of other mimosa species, *Acacia confusa* (EC_{50} = 0.088 mg/ml) [11], although the FRAP antioxidant capacity was lower (5890 nmolAAE/mg).

For waste forestry biomass, the highest total phenols content and antioxidant properties were obtained for the 50% methanol extracts. The extract antioxidant properties increased in the order: ethanol < methanol < water < 50% ethanol < 50% methanol. Comparing the results with those obtained for materials similar to waste forestry biomass, a mixture of different parts of pine and eucalyptus species, total phenols content were higher than those reported for pine sawdust methanol extracts (1.04–11.20 g GAE/100 g extract) [4] and of the same order as that reported for eucalyptus bark extracts in 50% methanol (20.19 g GAE/100 g extract) [6]. However, the FRAP antioxidant activity was lower than that found for eucalyptus bark extracts (2199 nmol AAE/mg extract) [6].

Comparing the three waste materials studied, the highest values for total phenols content, FRAP antioxidant capacity and ABTS radical scavenging capacity were obtained for the ethanolic extracts of mimosa wood whereas the highest DPPH radical scavenging capacity was found for the 50% methanol extracts of waste forestry biomass. As an example, fig. 1 shows the DPPH

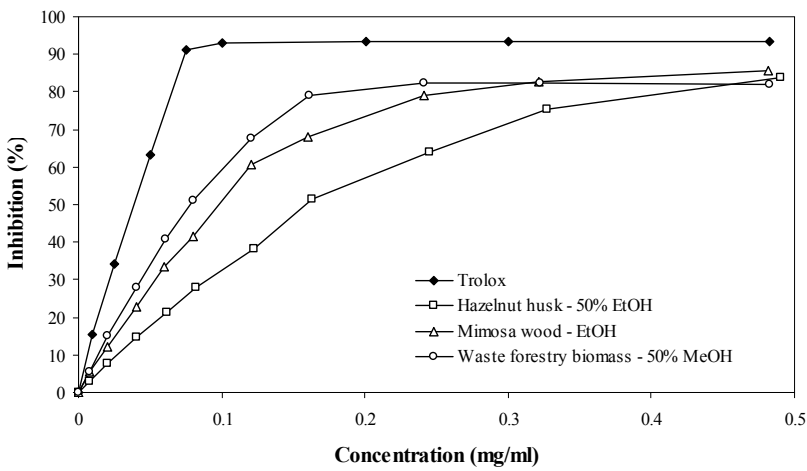

Figure 1: Scavenging activity on DPPH radical of the extracts.

radical inhibition (%) of the extracts obtained with the best solvent selected for each material together with the DPPH scavenging capacity of Trolox, the synthetic antioxidant used as reference. The capacity of the extracts to inhibit the DPPH radical was dependent on the extract concentration and all materials achieved a maximum inhibition of 80%. All the extracts showed lower DPPH scavenging capacity than Trolox and increased in the order hazelnut husks-50% EtOH<mimosa wood-EtOH<waste forestry biomass-50% MeOH.

3.4 Relationship between extract properties

Antioxidant properties of the extracts were related with their phenolic content. Thus, extracts with high total phenols content showed high antioxidant capacity measured by the three methods tested in this work. Analysing together the results obtained for hazelnut husks, mimosa wood and waste forestry biomass, linear relationships were found between TPC and FRAP antioxidant activity (R^2 = 0.8726, $p<0.001$)), TPC and DPPH scavenging activity (R^2 = 0.7748, $p<0.001$) and TPC and ABTS scavenging activity (R^2 = 0.9625, $p<0.001$). The relationships between the different antioxidant assays were also investigated and high linear correlations were found between FRAP antioxidant capacity and ABTS scavenging ability (R^2 = 0.8592, $p<0.001$) and between DPPH and ABTS methods (R^2 = 0.8506, $p<0.001$). FRAP and DPPH assays were the worst correlated (R^2 = 0.6330, $p<0.001$). Similar relationships were found for other plant materials [3, 7].

3.5 Characterization of the extracts

The extracts with the best antioxidant properties for each material (hazelnut husks – 50% ethanol; mimosa wood – ethanol; waste forestry biomass – 50% methanol) were analyzed by RP-HPLC-ESI-TOF mass spectrometry in order to identify the phenolic compounds responsible for their antioxidant activity. Fig. 2 shows the HPLC chromatograms of the selected extracts and the identified compounds are presented in table 3.

Five phenolic compounds were identified in hazelnut husk extract by comparison with the molecular weight and retention time of standard compounds: (-)-gallic acid, (-)-gallocatechin, (+)-catechin, ellagic acid and quercetin-3-O-rhamnoside. Protocatechuic acid (m/z 153) may also be present based on its molecular weight and its presence in hazelnut skin [12] also supports it. The presence of (+)-catechin, (-)-epicatechin, ellagic acid and quercetin-3-O-rhamnoside was confirmed in mimosa wood extract. Mono and di-galloyl glucose, (-)-gallic acid, (+)-catechin, (-)-epicatechin, ellagic acid, quercetin-3-β-D-glucoside and quercetin-3-O-rhamnoside were identified in waste forestry biomass extract. Other compounds to be considered based on their molecular weight were protocatechuic acid (m/z 153), chlorogenic acid (m/z 353), and naringenin (m/z 270), found in *Eucalyptus globulus* bark extracts [13] and limonene (m/z 135), myrcene (m/z 135), α-terpinolene (m/z 135), α-pinene (m/z 135) and β-pinene (m/z 135), found in *Pinus radiata* wood [14].

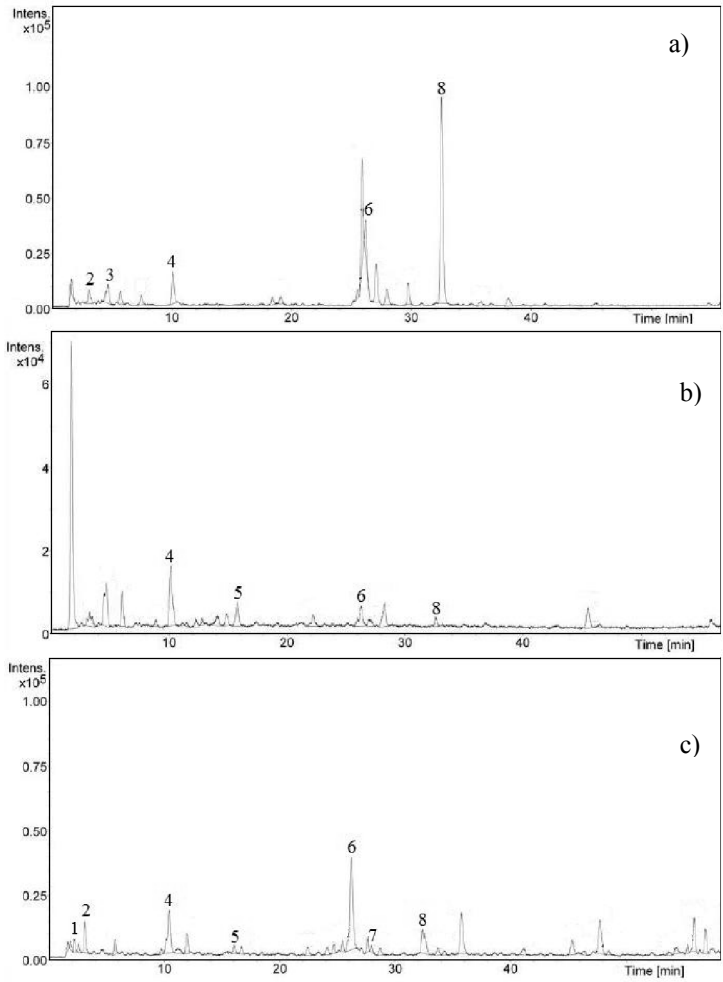

Figure 2: HPLC chromatograms of hazelnut husks (a), mimosa wood (b) and waste forestry biomass (c) extracts.

3.6 Molecular weight distribution by GPC

For each material the extract with the best antioxidant properties was analyzed by GPC to obtain the molecular weight distribution. The number (M_n) and weight (M_w) average molecular weights and the polydispersity index ($D = M_w/M_n$) were calculated and are shown in table 4. The M_n value of mimosa wood extract was significantly higher than those of the other materials whereas M_w hardly varied.

Table 3: Phenolic compounds identified in hazelnut husks (HH), mimosa wood (MW) and waste forestry biomass (WFB) extracts.

Peak	Compound	$[M-H]^-$ (m/z)	Retention time (min) (a.s.)	HH	MW	WFB
1	Mono, di-galloyl glucose	331/481	2.1	-	-	X
2	(-)-Gallic acid	169	3.0	X	-	X
3	(-)-Gallocatechin	305	4.7	X	-	-
4	(+)-Catechin	289	10.3	X	X	X
5	(-)-Epicatechin	289	15.9	-	X	X
6	Ellagic acid	301	26.4	X	X	X
7	Quercetin-3-β-D-glucoside	463	27.9	-	-	X
8	Quercetin-3-O-rhamnoside	447	32.6	X	X	X

(a.s.): according to the standard; (X): compound present in the extract; (-) compound that is not present in the extract.

Table 4: Average molecular weights and polydispersity index for hazelnut husks (HH), mimosa wood (MW) and waste forestry biomass (WFB) extracts.

	M_n (Da)	M_w (Da)	D
HH - 50% EtOH	512 ± 1	2625 ± 8	5.13 ± 0.03
MW – EtOH	835 ± 0	2476 ± 6	2.97 ± 0.01
WFB – 50% MeOH	640 ± 1	2706 ± 57	4.28 ± 0.09

The molecular weight distribution curves (fig. 3) showed a clearly defined peak with a maximum at 144 Da for all the materials, corresponding to low molecular weight compounds. In the medium range, the hazelnut husk extract showed a maximum at 798 Da, the mimosa wood extract at 537 Da and the waste forestry biomass extract at 630 Da. In the range of high molecular weights a maximum at 1270, 1350 and 1180 Da was obtained for hazelnut husks, mimosa wood and waste forestry biomass extracts, respectively.

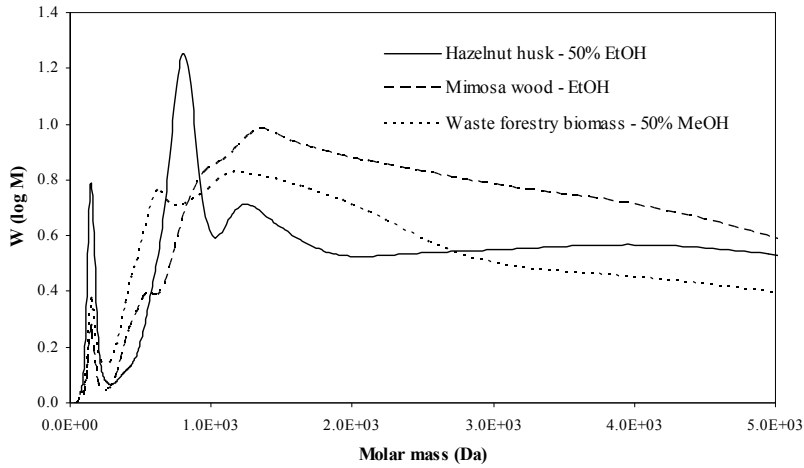

Figure 3: Molecular weight distribution curves of the extracts obtained by GPC.

4 Conclusions

For the waste material analysed, hazelnut husks, mimosa wood and waste forestry biomass, the influence of the solvent used (water, ethanol, methanol and their 50% aqueous solutions) on extraction yield and extract antioxidant properties was demonstrated. The best extracts properties were obtained using 50% ethanol for hazelnut husks, pure ethanol for mimosa wood and 50% methanol for waste forestry biomass. The highest total phenols content, FRAP antioxidant capacity and ABTS radical scavenging capacity were obtained for the ethanolic extracts of mimosa wood whereas 50% methanol extracts of waste forestry biomass showed the highest DPPH radical scavenging capacity. Various phenolic compounds with antioxidant capacity were identified in the extracts.

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References

[1] Moure, A., Cruz, J.M., Franco, D., Dominguez, H., Sineiro, J., Dominguez, H., Núñez, M.J., Parajó, J.C. Natural antioxidants from residual sources. *Food Chem.,* **72**, pp. 145-171, 2001.

[2] Contini, M., Baccelloni, S., Massantini, R., Anelli, G. Extraction of natural antioxidants from hazelnut (*Corylus avellana* L.) shell and skin wastes by long maceration at room temperature. *Food Chem*., **110**, pp. 659-669, 2008.
[3] Dudonné, S., Vitrac, X., Coutière, P., Woillez, M., Mérillon, J.M. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J. Agric. Food Chem.,* **57**, pp. 1768–1774, 2009.
[4] Pinelo, M., Rubilar, M., Sineiro, J., Núñez, M.J. Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*), *Food Chem*., **85**, pp. 267-273, 2004.
[5] Monagas, M., Garrido, I., Lebrón-Aguilar, R., Gómez-Cordovés, M.C., Rybarczyk, A., Amarowick, R., Bartolomé, B. Comparative flavan-3-ol profile and antioxidant capacity of roasted peanut, hazelnut and almond skins. *J. Agric. Food Chem*., **57**, pp. 10590-10599, 2009.
[6] Vázquez, G., Fontenla, E., Santos, J., Freire, M.S., González-Álvarez, J., Antorrena, G. Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts. *Ind. Crops Prod*., **28**, pp. 279-285, 2008.
[7] Vázquez, G., Fernández-Agúllo, A., Gómez-Castro, C., Freire, M.S., Antorrena, G., González-Álvarez, J. Response surface optimization of antioxidants extraction from chestnut (*Castanea sativa*) bur. *Ind. Crops Prod*. **35**, pp. 126-134, 2011.
[8] Maekawa, E., Ichizawa, T., Koshijima, T. An evaluation of the acid-soluble lignin determination in analyses of lignin by the sulphuric acid method. *J. Wood Chem. Technol*., **9**, pp. 549–567, 1989.
[9] Pereira, H. Variability in the chemical composition of plantation eucalypts (*Eucalyptus globulus* Labill). *Wood Fiber Sci*., **20**, pp. 82–90, 1988.
[10] Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem*., **28**, pp. 350–356, 1956.
[11] Wei, S.D., Zhou, H.D., Lin, Y.M., Liao, M.M., Chai, W.C. MALDI-TOF MS analysis of condensed tannins with potent antioxidant activity from the leaf, stem bark and root bark of *acacia confusa*. *Molecules*, **15**, pp. 4369-4381, 2010.
[12] Del Rio, D., Calani, L., Dall'Asta, M., Brighenti, F. Polyphenolic composition of hazelnut skin. *J. Agric. Food Chem*., **59**, pp. 9935–9941, 2011.
[13] Santos, S.A.O., Freire, C.S.R, Domingues, M.R.M., Silvestre, A.J.D., Neto, C.P. Characterization of phenolic components in polar extracts of *Eucalyptus globulus* labill. bark by high-performance liquid chromatography mass spectrometry. *J. Agric. Food Chem*., **59**, pp. 9386–9393, 2011.
[14] McDonald, A.G., Steward, D., Franich, R.A. Monoterpene composition of radiata pine (*Pinus radiata* D.Don) sapwood from a 13 year old progeny trial. *Holz Roh Werkst*, **57**, pp. 301–302, 1999.