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Abstract: Exhausted woods represent a byproduct of tannin industrial production processes and their possible exploitation as a source of antioxidant compounds has remained virtually unexplored. We herein report the characterization of the antioxidant and other properties of practical interest of exhausted chestnut wood and quebracho wood, together with those of a chestnut wood fiber, produced from steamed exhausted chestnut wood. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing/antioxidant power (FRAP) assays indicated good antioxidant properties for all the materials investigated, with exhausted chestnut wood, and, even more, chestnut wood fiber exhibiting the highest activity. High efficiency was observed also in the superoxide scavenging assay. An increase of the antioxidant potency was observed for both exhausted woods and chestnut wood fiber following activation by hydrolytic treatment, with an up to three-fold lowering of the EC50 values in the DPPH assay. On the other hand, exhausted quebracho wood was particularly effective as a nitrogen oxides (NOx) scavenger. The three materials proved able to adsorb methylene blue chosen as a model of organic pollutant and to remove highly toxic heavy metal ions like cadmium from aqueous solutions, with increase of the activity following the hydrolytic activation. These results open new perspectives toward the exploitation of exhausted woods as antioxidants, e.g., for active packaging, or as components of filtering membranes for remediation of polluted waters.

Keywords: agri-food waste; exhausted wood; antioxidant; DPPH assay; FRAP assay; tannins; heavy metals; methylene blue; nitric oxides; acid hydrolysis

1. Introduction

In the light of their marked antioxidant properties, phenolic polymers from natural sources have been the focus of increasing interest as sustainable functional materials for the wide range of potential applications, for example as biocompatible materials for biomedical devices or as active components in functional food packaging [1–7]. Indeed, phenolic polymers display manifold advantages over their monomers, including higher stability properties thus offering easier handling and processing, as well as lower solubility reducing tendency to be released [4,8,9].

Among natural phenolic polymers, tannins occupy a prime position given the well-established antimicrobial and protein binding properties [1,10–14]. Tannins are traditionally ranked into the
hydrolyzable and the condensed or non-hydrolyzable classes. *Castanea sativa* wood extract, commonly known as chestnut tannin, is composed mainly of hydrolyzable ellagitannins such as castalagin and its isomer vescalagin, whereas quebracho tannin, extracted from the hardwood of *Shinopsis balansae/lorentzii*, comprise mainly condensed tannins of which linear profisetinidins represent the major constituents (Figure 1) [1,15,16].

![Castalagin/vescalagin](image)

**Figure 1.** Representative structures of the main tannins occurring in chestnut and quebracho wood.

Both chestnut and quebracho tannins are commonly employed not only in the tanning industry, but also in the oenological, cosmetic, and pharmaceutical field as well as in products for animal feed [1,17–23]. Tannin extraction from wood is generally performed in hot water. The residual wood biomasses (exhausted woods), a by-product of the tannin extraction process, may be taken as renewable sources in their own right, since the wood has been treated only with hot water at high pressure, a treatment that is not expected to result in any significant change of the structural components. These exhausted woods are commonly used in the production of pellets for heating and energy production. The isolation of cellulose nanocrystals to be used e.g., as nanofillers for polymer composites from the exhausted acacia bark, obtained after the industrial process of extracting tannin, has also been reported [24]. However, the possible exploitation as antioxidant materials has remained virtually unexplored.

We report herein the characterization of the antioxidant properties of exhausted chestnut and quebracho wood, together with those of a chestnut wood fiber produced from steamed exhausted chestnut wood. The materials investigated are shown in Figure 2. For comparison, the corresponding fresh woods and tannins were investigated as well. Exhausted woods and chestnut wood fiber were also tested for their ability to remove environmental pollutants, either organic compounds or toxic heavy metals. Based on recent findings showing that acid hydrolytic treatment of natural phenolic polymers or wastes leads to materials with potent antioxidant efficiency [4,25,26], we also investigated the properties of the exhausted woods and chestnut wood fiber materials obtained by such treatment in comparison with those of the untreated materials, showing in most cases a significant enhancement of the activity. The results obtained open new perspectives for the exploitation of these by-products e.g., in active packaging or for remediation of polluted waters.
2. Materials and Methods

2.1. General Experimental Methods

Chestnut and quebracho exhausted and fresh woods, chestnut wood fiber as well as chestnut and quebracho tannins were provided by Silvateam (Via Torre, S. Michele Mondovì, Cuneo, Italy). In particular, fresh chestnut and quebracho woods were obtained from hardwood of 

\( \text{Castanea sativa} \) and \( \text{Schinopsis lorentzii} \) respectively, and reduced in chips of ca. 1–3 cm. Tannins were obtained by soaking the wood chips in autoclaves with water, at 120 °C, under pressure; the extracts thus obtained were concentrated with a multiple-effect evaporator under vacuum until ca. 50% water was removed, and tannin powder finally obtained by spray-drying. Residual chips after the extraction were stored as exhausted woods. Chestnut wood fiber was obtained from chestnut exhausted wood after drying in oven overnight at 60 °C and milling to obtain <250 μm particles.

2,2'-Diphenyl-1-picrylhydrazyl (DPPH), iron (III) chloride (97%), 2,4,6-tris(2-pirydyl)-s-triazine (TPTZ) (≥98%), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (97%), ethylenediaminetetraacetic acid (EDTA) (>99%), nitroblue tetrazolium (NBT) chloride (98%), pyrogallol (≥98%), quercetin (≥95%), fluorescein, 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) (97%), Folin & Ciocalteu’s phenol reagent (2N), gallic acid (≥97.5%), activated carbon, sodium nitrite (≥97.0%), \( \text{N-(1-naphthyl)ethylenediamine dihydrochloride (≥98%)} \), sulfanilamide (≥99%), methylene blue (MB), cadmium carbonate (99%), and nitric acid (≥69% v/v, TraceSELECT® water solution) were obtained from Sigma-Aldrich (Milan, Italy) and used as obtained.

UV-Vis spectra were performed using a HewlettPackard 8453 Agilent spectrophotometer. Fluorescence spectra were record on a HORIBA Jobin Yvon Inc. FluoroMax®-4 spectrofluorometer.

For metal removal experiments, 1% HNO₃, 0.1 M HCl and 0.01 M phosphate buffer (pH 7.0) were prepared using ultrapure deionized water with conductivity <0.06 μS/cm. All glassware used were carefully washed first with a 1% HNO₃ solution and then with ultrapure deionized water. Metal analysis was carried out on an inductively coupled plasma mass spectrometry (ICP-MS) instrument Aurora M90 model by Bruker.

2.2. Hydrolytic Treatment

The proper material (3 g) was treated with 70 mL of 6 M HCl under stirring at 100 °C overnight [4,26]. After cooling at room temperature, the mixture was centrifuged (8247×g, room temperature, 15 min) and the precipitate washed with water until neutrality and freeze dried. The recovery yields were 58% (chestnut wood fiber), 68% (exhausted chestnut wood) and 83% (quebracho exhausted wood).

2.3. DPPH Assay

To a 0.2 mM methanolic solution of DPPH wood or tannin powders were added (final dose 0.03–1.5 mg/mL) and after 10 min under stirring at room temperature the absorbance of the solution...
at 515 nm was measured [27,28]. Experiments were run in triplicate. Trolox was used as reference antioxidant.

2.4. Ferric Reducing/Antioxidant Power (FRAP) Assay

To 0.3 M acetate buffer (pH 3.6) containing 1.7 mM FeCl₃ and 0.83 mM TPTZ, wood or tannin powders (final dose 0.00625–0.3 mg/mL) were added and after 10 min under stirring at room temperature the absorbance of the solution at 593 nm was measured [29]. Results were expressed as Trolox equivalents. Experiments were run in triplicate.

2.5. Superoxide Scavenging Assay

Wood or tannin powders (final dose 0.0625–0.3 mg/mL) were added to 0.05 M ammonium hydrogen carbonate buffer (pH 9.3) containing 0.4 mM EDTA and 12 μM NBT, followed by a 20 mM pyrogallol solution in 0.05 mM HCl (final pyrogallol concentration 3.3 mM) [4,30]. The mixture was vigorously stirred for 5 min, after that absorbance at 596 nm was measured. Results were expressed as percentage of reduction of the absorbance at 596 nm of a control mixture run in the absence of sample. Experiments were run in triplicate.

2.6. Oxygen Radical Antioxidant Capacity (ORAC Assay)

Wood or tannin powders (final dose 0.075 mg/mL) were incubated in a 62.7 μM fluorescein solution in 75 mM phosphate buffer (pH 7.4) for 30 min, at 37 °C. A 153 mM AAPH solution was then added (final APPH concentration 19 mM) and after stirring at 37 °C for 45 min fluorescence was measured (λₑₓ = 485 nm, λₑₘ = 511 nm) [31]. Results were expressed as relative fluorescence intensity with respect to a control mixture run in the absence of APPH. Experiments were run in triplicate.

2.7. MB Adsorption Assay

Adsorption experiments were performed at room temperature by adding wood samples (0.2 mg/mL) to a 5 or 25 mg/L aqueous solution of MB. The mixtures were taken under stirring and after 30 min the absorbance at 654 nm was measured [32]. Activated carbon was used as reference material. Experiments were run in triplicate.

2.8. Nitric oxides (NOₓ) Scavenging Assay

A solution of sodium nitrite (1 M) in water was added to 10% sulfuric acid over 10 min [33]. The red orange gas that developed (0.2–0.6 mL) was withdrawn with a syringe and conveyed through a tip containing 5 mg of wood sample into 3 mL of Griess reagent (0.5% sulfanilamide and 0.05% N-(1-naphthyl)ethylenediamine dihydrochloride in 1.25% phosphoric acid) and the absorbance at 540 nm was measured. Experiments were run in triplicate.

2.9. Cd²⁺ Adsorption

A 1.5 mM stock solution of the heavy metal was prepared by dissolving 10 mg of cadmium carbonate in 39 mL of 0.1 M HCl. Prior to the adsorption experiments a 1.5 mg/mL suspension of each wood sample in 0.01 M phosphate buffer (pH 7.0) was obtained by homogenization in a Tenbroeck glass to glass homogenizer for 4 min. 0.7 mL of the wood suspensions and 0.1 mL of the metal solution were added to 10 mL of 0.01 M phosphate buffer at pH 7.0. After 2 h, the mixtures were filtered through a 0.45 μm nylon membrane, acidified by addition of 69% nitric acid (1:100 v/v), properly diluted with 1% nitric acid, and analyzed by ICP-MS [34]. A calibration curve was built with cadmium solutions at five different concentrations. For each binding experiment a blank experiment was planned in which the metal ion was added in the phosphate buffer and incubated for 2 h without addition of the wood sample. Experiments were run in triplicate.

2.10. Evaluation of the Solubility of Woods and Tannins in the Assay Media
Wood or tannin samples (3 mg) were added to methanol (20 mL), 0.3 M acetate buffer (pH 3.6) (20 mL), or water (15 mL), and taken under magnetic stirring. After 10 or 30 min the supernatants obtained after centrifugation (8247 \( \times \) g, room temperature, 15 min) were analyzed by UV-Vis spectrophotometry.

2.11. Determination of the Amount of Tannins in the Wood Samples

Wood or tannin samples (10 mg) were stirred in 1 mL of a 1:1 \( v/v \) acetone/water mixture containing 1% acetic acid [35]. After 60 min the supernatants obtained after centrifugation (3534 \( \times \) g, room temperature, 20 min) were analyzed by UV-Vis spectrophotometry after 1:500 \( v/v \) dilution in methanol. The amount of tannins in each wood sample was determined by comparison of the absorbance at 269 nm (chestnut-derived samples) or 280 nm (quebracho-derived samples) with that measured for chestnut or quebracho tannins, respectively.

2.12. Measurement of Total Phenolic Content (TPC)

Wood or tannin samples (10 mg) were stirred in DMSO (1 mL) for 1 h. After centrifugation (3534 \( \times \) g, room temperature, 20 min) 1–50 \( \mu \)L of the supernatant were added to 1.4 mL of water followed by 0.3 mL of a 75 g/L \( \text{Na}_2\text{CO}_3 \) solution and 0.1 mL of Folin & Ciocalteu’s reagent. After 30 min incubation at 40 °C, absorbance at 765 nm was measured [36]. Gallic acid was used as reference compound. Experiments were run in triplicate.

3. Results and Discussion

3.1. Antioxidant Properties of Exhausted Woods

In a first series of experiments the antioxidant properties of chestnut wood fiber and exhausted chestnut and quebracho woods were investigated with respect to the corresponding fresh woods and tannins by widely used assays, i.e. DPPH, FRAP, superoxide scavenging, and ORAC assays following the “QUENCHER” method which allows one to measure the efficiency of electron transfer processes from a solid antioxidant [27–31].

3.1.1. DPPH and FRAP Assays

Table 1 reports the EC\(_{50}\) value, which is the dose of the material at which a 50% DPPH reduction is observed, determined for tannins and wood samples in the DPPH assay. For comparison data for the reference antioxidant Trolox are also reported.

<table>
<thead>
<tr>
<th>Sample</th>
<th>EC(_{50}) (mg/mL) (^1) (DPPH Assay)</th>
<th>Trolox Equivalents (FRAP Assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chestnut wood fiber</td>
<td>0.054 ± 0.003</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Exhausted chestnut wood</td>
<td>0.436 ± 0.003</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Fresh chestnut wood</td>
<td>0.128 ± 0.003</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Chestnut tannins</td>
<td>0.019 ± 0.002</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Exhausted quebracho wood</td>
<td>1.14 ± 0.04</td>
<td>0.051 ± 0.005</td>
</tr>
<tr>
<td>Fresh quebracho wood</td>
<td>0.0990 ± 0.0007</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Quebracho tannins</td>
<td>0.026 ± 0.002</td>
<td>0.47 ± 0.03</td>
</tr>
<tr>
<td>Trolox</td>
<td>0.015 ± 0.001</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Reported are the mean ± SD values of at least three experiments. \(^2\) EC\(_{50}\) is the dose of the material at which a 50% DPPH reduction is observed.

Among the waste materials, chestnut wood fiber displayed the most promising DPPH-reducing ability, with an EC\(_{50}\) value of 0.054 mg/mL that compares well with that of Trolox (3.6-fold higher). Also quebracho and particularly chestnut exhausted wood exhibited quite low EC\(_{50}\) values, much lower than those reported for other agro-food wastes, such as spent coffee grounds (EC\(_{50}\) = 5.00 mg/mL) [4]. As expected, higher antioxidant activities were exhibited by fresh wood samples, still
containing tannins, and tannins themselves, characterized by EC₅₀ values approaching that of Trolox.

The marked differences observed in the antioxidant activity may be interpreted considering the solubility of the materials in the assay medium. Spectra shown in Figure 3a clearly indicate release of UV absorbing species in the case of pure tannins, chestnut wood fiber, and fresh woods, whereas exhausted woods do not give rise to appreciable absorbance.

The results of the FRAP assay (Table 1) looked less encouraging than those obtained in the DPPH assay, with all the waste materials exhibiting an iron(III)-reducing activity far lower than Trolox (Trolox equivalents <<1). Only chestnut tannins showed a satisfactory antioxidant power in this assay, whereas the fresh wood samples as well as quebracho tannins performed much less. Here again the antioxidant activity parallels fairly well the solubility in the medium used for the FRAP assay (Figure 3b).

![Figure 3. UV-Vis spectra of wood and tannin samples (0.15 mg/mL) in methanol (a) or 0.3 M acetate buffer (pH = 3.6) (b).](image)

To obtain information about the compounds responsible for the antioxidant properties observed, the amounts of tannins and the TPC were determined for each wood sample (Table 2). As shown in Figure 4, a good linear correlation was found between Trolox equivalents determined in the FRAP assay and the tannin content (for both the hydrolyzable and non-hydrolyzable class), but not TPC ($R^2 = 0.59$), pointing to residual tannins as the main determinants of the iron-reducing properties. On the contrary, DPPH reducing ability was not apparently related to either TPC ($R^2 = 0.37$) or tannin content ($R^2 = 0.33$ and 0.50 for chestnut and quebracho-derived samples, respectively), suggesting that several factors (e.g., relative solubility in the assay medium) might be involved in the different activities observed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tannins (% w/w)</th>
<th>TPC (mg gallic acid/g of sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chestnut wood fiber</td>
<td>19</td>
<td>151 ± 17</td>
</tr>
<tr>
<td>Exhausted chestnut wood</td>
<td>11</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>Fresh chestnut wood</td>
<td>21</td>
<td>153 ± 9</td>
</tr>
<tr>
<td>Chestnut tannins</td>
<td>100</td>
<td>457 ± 59</td>
</tr>
<tr>
<td>Exhausted quebracho wood</td>
<td>28</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>Fresh quebracho wood</td>
<td>43</td>
<td>194 ± 8</td>
</tr>
<tr>
<td>Quebracho tannins</td>
<td>100</td>
<td>550 ± 99</td>
</tr>
</tbody>
</table>

1 Reported are the mean values of at least three experiments (SD ≤ 5%). 2 Reported are the mean values ± SD of at least three experiments.
Figure 4. Correlation between tannin content and Fe\(^{3+}\)-reducing activity of tannin and wood samples. (a) Chestnut-derived samples; (b) Quebracho-derived samples.

The exhausted woods and the chestnut wood fiber that have been subjected to the hydrolytic treatment according to the protocol developed in previous studies [4,25,26] were then evaluated for their antioxidant activity by the DPPH and FRAP assays. Data shown in Figure 5a indicate for both exhausted quebracho and chestnut woods a decrease of EC\(_{50}\) values in the range of 25%–30% compared to the values obtained for the untreated materials. By contrast no significant increase of the activity was observed in the FRAP assay following the hydrolytic treatment, apart for chestnut wood fiber, which exhibited a two-fold increase in Trolox eqs (Figure 5b).

Figure 5. Antioxidant properties of exhausted wood samples before and after hydrolytic activation. (a) DPPH assay; (b) ferric reducing/antioxidant power (FRAP) assay. Reported are the mean ± SD values of at least three experiments.

3.1.2. Superoxide Scavenging Assay

A very high efficiency, compared to other agri-food waste products such as spent coffee grounds [4], was observed in the superoxide scavenging assay (Figure 6), with tannins and fresh woods being always the most active samples, although in the case of chestnut-derived materials both the exhausted woods exhibited an activity comparable to that of the native sample. Only a modest correlation (\(R^2 = 0.90\) and 0.82 for chestnut and quebracho-derived samples, respectively) was found between the percentage of superoxide scavenging and the tannin content, whereas no correlation was found with TCP. Moreover, no significant improvement in the scavenging ability was detected in exhausted woods subjected to the hydrolytic treatment.
Figure 6. Superoxide scavenging activity of tannins and wood samples. Reported are the mean ± SD values of at least three experiments.

3.1.3. ORAC Assay

The relative fluorescence intensities determined in the ORAC assay are reported in Table 3. In this case major differences were apparent between tannins/fresh woods and exhausted samples, with the first ones almost totally inhibiting fluorescein oxidation and the second ones being completely inactive, with the only exception of chestnut wood fiber, which showed an activity comparable to that of fresh chestnut wood. Notably, in this case a good linear correlation ($R^2 = 0.95$) was found between the antioxidant activity of the wood samples and TPC; on the contrary no significant correlation was found with the amount of residual tannins. No effect of the hydrolytic treatment was apparent either in this assay.

Table 3. Activity of tannins and wood samples in the oxygen radical antioxidant capacity (ORAC) assay.1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relative fluorescence intensity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>0.22</td>
</tr>
<tr>
<td>Chestnut wood fiber</td>
<td>86</td>
</tr>
<tr>
<td>Exhausted chestnut wood</td>
<td>0.5</td>
</tr>
<tr>
<td>Fresh chestnut wood</td>
<td>89</td>
</tr>
<tr>
<td>Chestnut tannins</td>
<td>81</td>
</tr>
<tr>
<td>Exhausted quebracho wood</td>
<td>0.2</td>
</tr>
<tr>
<td>Fresh quebracho wood</td>
<td>90</td>
</tr>
<tr>
<td>Quebracho tannins</td>
<td>100</td>
</tr>
</tbody>
</table>

1 Reported are the mean values of at least three experiments (SD ≤ 5%). 2Mixture containing only fluorescein and APPH, without any antioxidant.

The results of the antioxidant assays further add to the potential of wood fiber as a reinforcement in polymers [37,38] or as an ecological-friendly medium for horticultural practice to increase the antioxidant activity of fruit and vegetables [39].
3.2. Pollutant Adsorption Properties of Exhausted Woods

In a further series of experiments the adsorption capacity of the exhausted woods toward various environmental pollutants was evaluated. These included MB, as a model organic dye, \(\text{NO}_x\), that is nitric oxide (NO) and nitrogen dioxide (\(\text{NO}_2\)) which are reactive nitrogen species commonly present in cigarette smoke and exhaust gases able to induce oxidative and nitrosative stress in humans, and cadmium ions (\(\text{Cd}^{2+}\)), as a model of toxic heavy metals.

3.2.1. MB Adsorption Assay

Table 4 reports the percentages of MB adsorption by the wood samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MB adsorption (%)</th>
<th>(\text{NO}_x) scavenging (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chestnut wood fiber</td>
<td>100</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>Exhausted chestnut wood</td>
<td>73 ± 3</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>Fresh chestnut wood</td>
<td>79 ± 1</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>Exhausted quebracho wood</td>
<td>77 ± 1</td>
<td>79 ± 1</td>
</tr>
<tr>
<td>Fresh quebracho wood</td>
<td>77 ± 2</td>
<td>86 ± 5</td>
</tr>
</tbody>
</table>

1. Reported are the mean ± SD values of at least three experiments. 2. Determined with methylene blue (MB) at 5 mg/L. 3. Determined with 0.6 mL of gas.

Both exhausted chestnut and quebracho woods proved to be very efficient at a dose of 0.2 mg/mL, and their adsorption properties were comparable to those exhibited by the fresh wood samples. Indeed, UV-Vis spectrophotometric analysis (Figure 7) indicated a lower solubility in the assay medium (water) of chestnut wood fiber and exhausted woods compared to the fresh samples, a feature that further adds to the potential of these materials for removal of organic compounds from waste waters.

![Figure 7. UV-Vis spectra of wood samples (0.2 mg/mL) in water.](image_url)

A 20%-30% dye removal was still observed when wood samples were added to a more concentrated MB solution (25 mg/mL) (Figure 8). For comparison, under the same conditions a 70% MB removal was obtained with activated carbon, taken as a reference material. In contrast to what observed in the antioxidant assays, the MB adsorption ability of the exhausted woods did not change appreciably following to acid treatment.
3.2.2. NO. Scavenging Assay

All the samples examined led to >70% scavenging when 0.2 mL of NO\textsubscript{x} gases were passed through 5 mg of wood (data not shown). The percentages of NO\textsubscript{x} scavenging determined in the experiments run with 0.6 mL of gas are reported in Table 4, from which the superior ability of quebracho woods stands out compared to chestnut woods. These data would suggest a higher trapping efficiency of condensed tannins with respect to hydrolyzable tannins that could likely be ascribed to the reactivity of the resorcinol moieties of profisetinidins toward electrophiles like nitric oxides [40]. As observed above for MB adsorption, also in this case fresh woods were only slightly more active than exhausted samples. Notably, further to acid treatment a lower efficiency was observed for most of the tested woods (not shown), pointing to a role of the hydrolyzable cellulosic matrix in NO\textsubscript{x} scavenging.

3.2.3. Cd\textsuperscript{2+} Removal Assay

In a last series of experiments the ability of wood samples to remove heavy metals from aqueous solutions was investigated using Cd\textsuperscript{2+} as model ions. The assay was performed at pH 7.0, based on previous observations showing that natural phenolic polymers do not exhibit significant chelating properties at acidic pH [34]. In this case fresh woods were not evaluated because of the release of significant amounts of material under the testing conditions, likely due to solubilization of residual tannins and/or other low molecular weight components.

Based on the data reported in Figure 9, chestnut wood fiber was again the most active among the waste materials, followed by exhausted quebracho wood. An increase in Cd\textsuperscript{2+} removal was observed following hydrolytic activation for all the woods examined.
4. Conclusions

Exhausted woods represent a largely available waste product of the tannin extraction process, whose practical exploitation has not yet been duly considered. As reported in the present paper, these materials exhibit good antioxidant properties in the DPPH and superoxide scavenging assay, and are also able to efficiently adsorb pollutants such as toxic gases, organic dyes and heavy metals. In particular, chestnut woods, containing mainly hydrolyzable tannins, were particularly active as antioxidants, whereas quebracho woods, characterized by the presence of condensed tannins, were found to be more effective as adsorbent materials, especially toward NO\textsubscript{x} fumes. Of particular interest is the enhancement of the antioxidant properties of exhausted woods and chestnut wood fiber following hydrolytic treatment, a methodology that had already been applied to materials containing lignins like spent coffee grounds [4] and the black sesame seed pigment [25], but also ellagitannins like pomegranate wastes [26]. An interpretation of such effects at a molecular level invoke aromatization and dehydration processes enhancing the hydrogen donor ability of the OH functionalities stemming from delocalization of the resulting phenoxy radical over a more conjugated molecular backbone [4,8]. On the other hand, from consideration of the moderate weight loss further to the hydrolytic treatment it cannot be argued that the observed activation effects may simply be ascribed to removal of cellulose or other inactive components of the materials.

Overall, these results would point to exhausted woods as novel potential functional additives to be used for example in active packaging, as food stabilizers against oxidative deterioration, or in filtering devices as pollutant removal agents.

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