Supporting Information

A novel enzymatic approach to develop a lignin-based adhesive for wool floor coverings

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Experimental

Laccase activity determination

Laccase activity was determined spectrophotometrically (Helios Gamma spectrophotometer from Thermo Electron Corp., USA) by monitoring the change of absorbance of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) upon enzymatic oxidation. One activity unit was defined as the amount of enzyme converting 1 μmol ABTS per min to its cation radical ($\varepsilon_{436nm} = 29\,300\ M^{-1}\ cm^{-1}$) in 0.1 M sodium acetate buffer pH 5 at 25 °C.

Laccase stability assays

50 μL laccase was incubated in 100 mL 0.1 M citrate-phosphate-borate buffer at different pHs and temperatures, using thermostatic baths. The residual activity values were expressed as a percentage of the initial enzyme activity (1277 U/mL). t0 was set as the time after addition of the enzyme and homogenisation of the solution (10 s).

FTIR analysis

Attenuated Total Reflectance (ATR) technique was used to perform FTIR analysis of lignin samples. Spectra were collected over the $500 - 4000\ cm^{-1}$ range by a Perkin Elmer Spectrum 100 FT-IR spectrometer, recording 32 scans at 4 cm^{-1} resolution for each spectrum.

Results and discussion

Laccase stability assays

Monitoring the stability of laccase is of crucial importance for its applicability under the operative conditions of adhesive production. Despite its relatively low redox potential compared to other fungal laccases, the laccase from *Mt*L shows higher stability to temperature and alkaline pH,[S1] Our interest was to use this enzyme at more alkaline condition (pH 6 - 12) than those previously investigated due to the better solubilisation of most industrial lignins with the increase of pH. The residual activity of *Mt*L remained between 80 and 90 % during the first 5 h of incubation at 25 ℃ in the pH range 6 – 11. After 18 h, the residual activity dropped to 70 % in the pH range 8 – 11, while at pH 12 the activity decreased to ~20 % (Fig. S1).
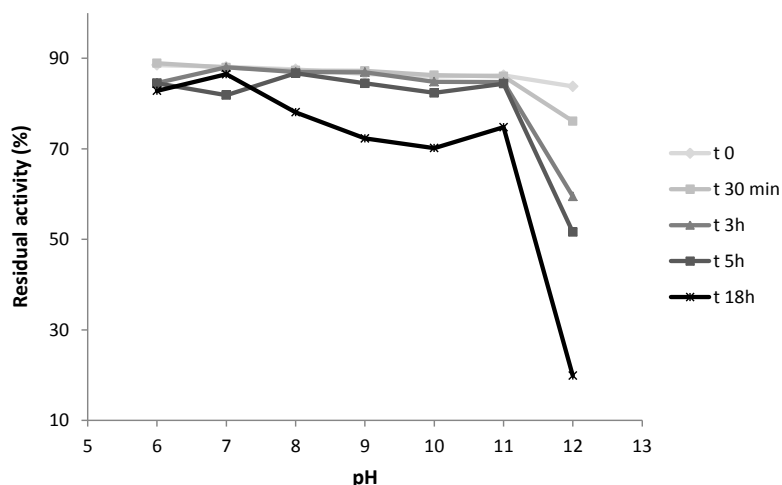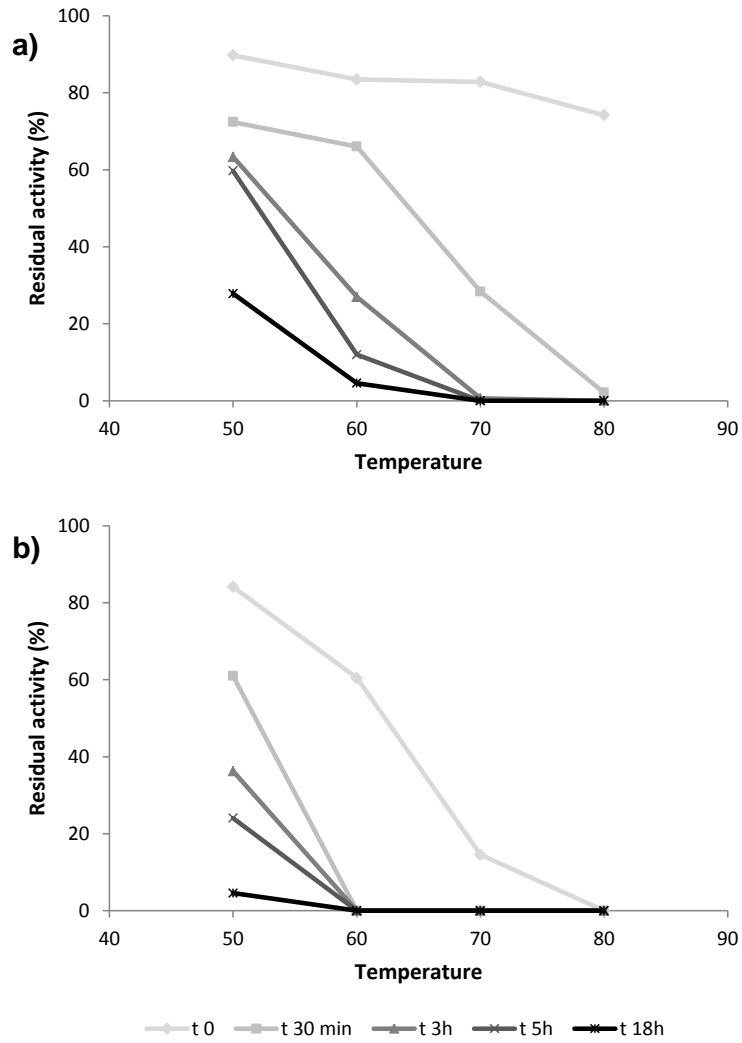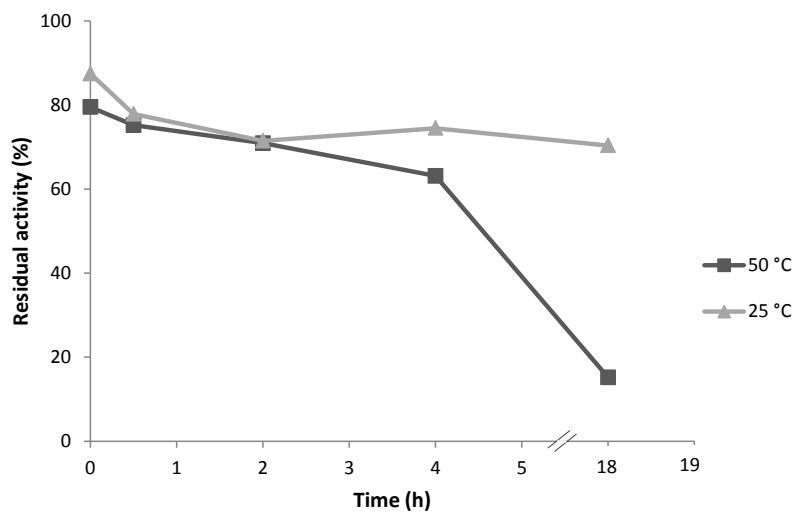

Fig. S1: Profiles of *Mt*L residual activity over 18 h in the pH range 6-12 at 25 °C

At pH 7 (the optimum pH according to the provider), the enzyme retained 60 % residual activity after 5 h at 50 °C, whereas after 5 h at 60 °C only ~10 % activity remained (Fig. S2a). The effect of temperature was more pronounced at pH 11 - the enzyme showed a gradual loss of activity at 50 °C, whereas it was rapidly inactivated at higher temperatures (Fig. S2b).

a)

b)

t 0 t 30 min t 3h t 5h t 18h

Fig. S2: Profiles of *Mt*L residual activity over 18 h in the temperature range 50-80 °C, at pH 7 (a) and 11 (b)


50 °C

25 °C

Fig. S3: Profiles of *Mt*L residual activity over 18 h in the presence of PEG (20 % v/v) at 25 and 50 °C

S4

The alkaline pH in the adhesive synthesis favours both the autopolymerization of DA and the deprotonation of the amine groups during the Michael addition reaction to quinone structures. Thus, the adhesive generation process was carried out at 50 °C and pH 8.5 according to the observed enzyme stability profiles.

FTIR analysis of lignin samples

The assignments of the peaks in the FTIR spectra (Fig. S4) was according to Faix (1992).[S2] The bands were normalized using Perkin Elmer Spectrum software against the band at 1514 cm^{-1} typical for the stretching of aromatic rings.
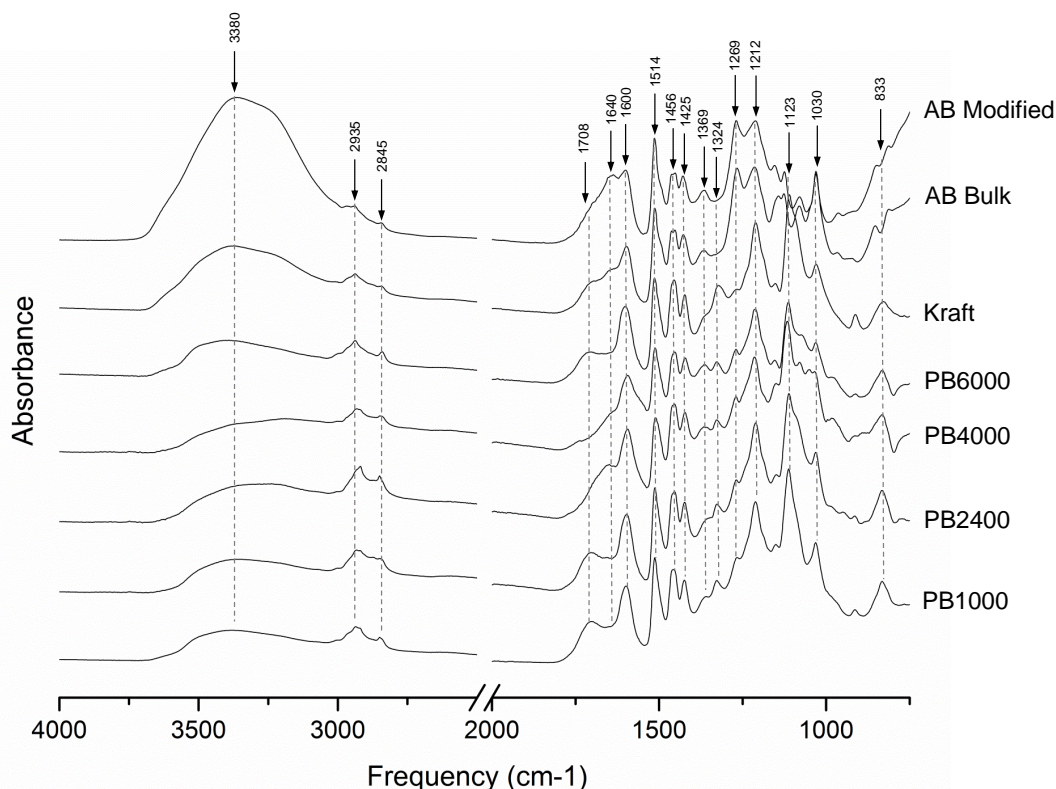
Fig. S4: FTIR spectra of the studied lignins

All studied lignins presented characteristic bands for aromatic ring vibration of the phenylpropane skeleton at 1600 cm^{-1} and at 1425 cm^{-1}, and a band at 1456 cm^{-1} related to C-H vibration of CH$_2$ and CH$_3$ groups. The band due to the presence of phenolic hydroxyl groups at 1369 cm^{-1} is more intense in AB Bulk and Modified lignins in good agreement with the spectrophotometric determinations.

A characteristic peak at 1326-1323 cm^{-1} for syringyl (S) ring and syringyl and guaiacyl (G) condensed ring appeared in the spectra of kraft and Protobind lignins. These lignins also showed strong signals: i) at 1123 cm^{-1}, attributed to both C-H deformation of the aromatic group of S units and C-O deformation in secondary alcohols, and ii) at 833 cm^{-1} associated with syringyl linkages.

AB Bulk and Modified lignins presented: i) a peak at 1269 cm^{-1}, typical for C-O and C=O groups stretching in G rings, ii) a predominant signal at 1030 cm^{-1} assigned to C-H deformation of the aromatic group of G units, C-O deformation

of primary alcohols and stretching of non-conjugated C=O. The strong vibration at 1212-1217 cm^{-1} is common for the spectra of all lignins and can be associated with C-C, C-O and C=O stretching. The OH stretching in phenolic and aliphatic structures appears in the range of 3100-3600 cm^{-1}, being particularly intense for AB Modified lignin.

The bands clustered around 2935 and 2845 cm^{-1} predominantly arise from C-H stretching in aromatic methoxyl groups, and in methyl and methylene groups of side chains. In the carbonyl region, a band at 1708 cm^{-1}, originating from unconjugated carbonyl/carboxyl stretching, is found for PB1000, PB2400, kraft and AB Bulk lignins. The signal at 1640 cm^{-1} related to the presence of conjugated carbonyl groups, appears as a peak for AB Modified and PB4000 lignins and as a shoulder for AB Bulk and PB6000 lignins.

Screening for suitable adhesive-generating lignins

Prior to producing the adhesive paste, the lignin solubility was assayed in: 50 mM Tris-HCl buffer pH 8.5, acetone, ethanol, PEG, and mixtures (80:20) of buffer and each of the organic solvents. Among the studied lignins, PB6000 was the most soluble (completely soluble in organic solvents and highly soluble in the alkaline buffer) in all tested media. Each lignin was employed for adhesive production in buffer and in the absence of AS. The adhesives obtained provided satisfactory bonding strength (LWF values between 60 and 75 N), bearing in mind that 30 N is the minimum strength requirement according to EN 1307 standard for the suitability of carpets for intensive commercial use. Nevertheless, after conditioning, all adhesives became brittle and released powder, apparently depending on lignin solubility in the buffer. The PB6000-based adhesive showed LWF = 75 N and the highest flexibility, therefore PB6000 lignin was selected for further trials.

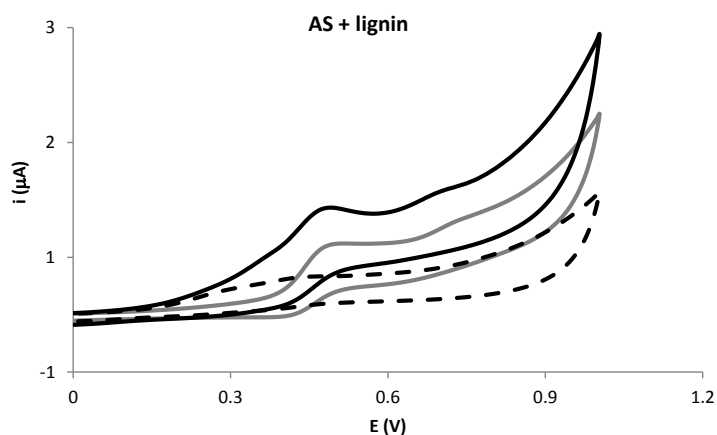**Cyclic voltammetry analysis**

Fig. S5: Cyclic voltammograms of 0.1 mM solution of AS (gray line), 0.05 mg/ml solution of lignin (dashed line), and AS plus lignin (black line). 100 mM Tris-HCl buffer pH 8.5. Scan rate 5mV/s

CVs at different scan rates were recorded for solutions containing two phenolic compounds in order to evaluate their reactivity towards each other (Fig. S6). Increasing the scan rate, the probability of the oxidised intermediates to undergo chemical reactions decreases. Thus, the comparison of CVs at low and high scan rates would give an indication whether the two compounds react with each other. Two anodic peaks corresponding to the oxidation potentials of DA and GA can be detected at high scan rates, while decreasing the scan rate, these peaks converged and shifted to lower potentials (Fig. S6a). This indicates the occurrence of a reaction between DA and GA yielding a product with a lower oxidation potential. CVs obtained for the solution containing DA and TA (Fig. S6b) showed an anodic peak at higher potential than the oxidation potentials of each compound when assayed alone. On the other hand, the lack of anodic peaks associated with their oxidation products was observed at high potentials. These findings support the occurrence of a reaction between the two compounds that yields a new product with different redox properties when compared to the initial compounds and their own oxidation products. Increasing the scan rate did not result in the appearance of oxidation peaks related to unreacted compounds, indicating that the reaction between them occurs faster than the reaction between DA and GA. CVs obtained for the solution containing TA and GA (Fig. S6c) showed at all scan rates the anodic waves characteristic for TA alone - the wave at the lower potential overlaps with that of GA. Increasing the scan rate, the intensity of the second oxidation signal becomes higher compared to the first one, suggesting that the formation of the second oxidation product of TA occurs with faster kinetics than the reaction with GA.
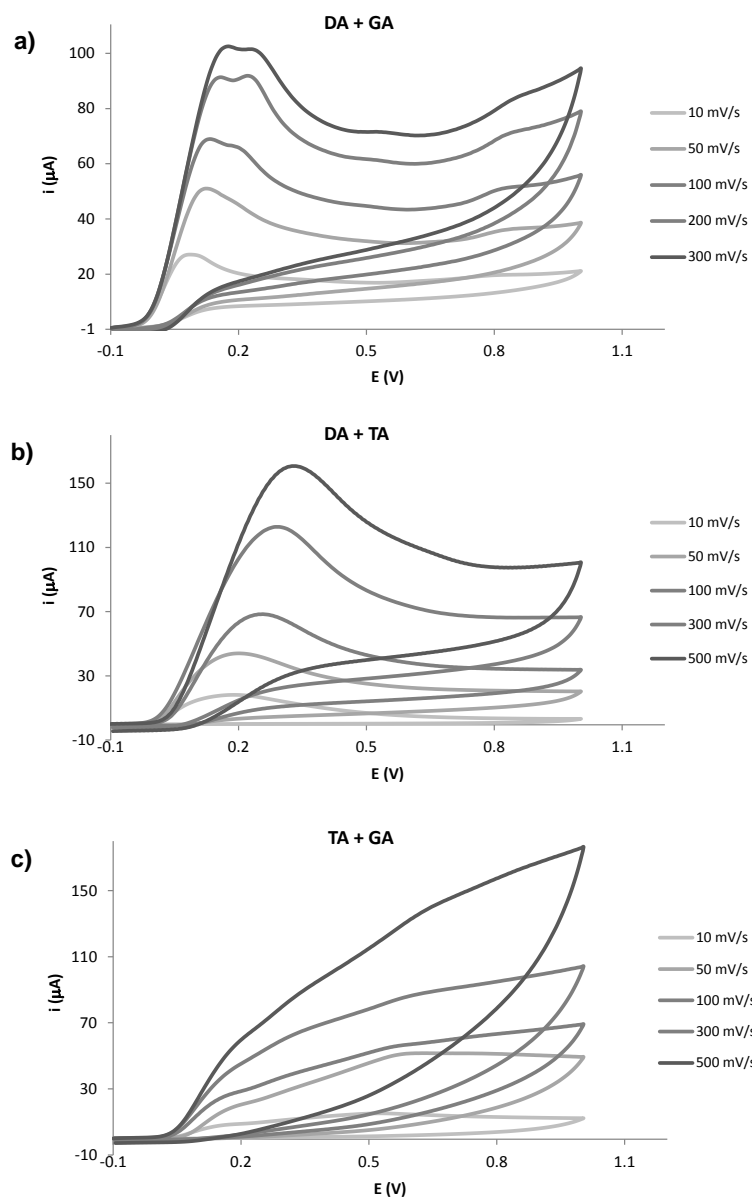
Fig. S6: Cyclic voltammograms recorded at different scan rates (10-500 mV/s) for solutions containing DA plus GA (a), DA plus TA (b) and TA plus GA (c), in 100 mM Tris-HCl buffer pH 8.5

References

S1 D. Ibarra, J. Romero, M. Martínez, A. Martínez and S. Camarero, *Enz. Microb. Technol.,* 2006, **39**, 1319-1327.

S2 O. Faix, in ed. S. Lin and C. Dence, Springer Berlin Heidelberg, 1992, pp.233-241.