

PRODUCTION OF POLYMER COMPOSITES USING LIGNIN OBTAINED BY HIGH YIELD WOOD EXTRACTION WITH GREEN SOLVENTS

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SUMMARY

Lignin is one of the major polymers present in lignocellulosic materials and one of the most abundant polymers in nature. In the production of cellulosic kraft pulp this aromatic polymer is often only used for energy production (about 98% of the lignin present in the black liquor is burnt), which is far from being the most adequate approach, considering the high potential of this polymer. Recent trends in biorefinery have tried to valorise lignin by its transformation into other value-added products. In this work, it will be presented the results of the lignin extraction from *Acacia dealbata* wood (an invasive tree species) using a selected ionic liquid (IL) and a deep eutectic solvent (DES). A Kraft lignin obtained from the black liquor of *Acacia dealbata* cooking and a commercial lignin from Sigma-Aldrich were used as references. The methods of lignin extraction and isolation using “green” solvents have been optimized in order to obtain high purity lignin(s) in a high yield. The results showed that it was possible to obtain yields of lignin recover approaching 100% for the more severe conditions of lignin extraction (140 °C; 24 h using IL + H₂O and 180 °C; 24 h using the DES). For less severe conditions of extraction (120 °C; 24 h and 140 °C; 8 h using IL + H₂O, and 160 °C; 24 h and 180 °C; 8 h using the DES), lignin yields were in the range of 68-81%. On the other hand, the isolated lignins showed total lignin (Klason lignin plus acid-soluble lignin) values between 83 and 93%, revealing high-purity lignins. Minor amounts of polysaccharides were found (less than 1%) in the isolated lignins. Lignins were then used in the production of composites with polybutylene adipate terephthalate (PBAT), a promising polymer for plastic production with good biodegradability and mechanical properties. It was found that lignin could improve stiffness in the composites with this polymer.

Keywords: bioresources, circular economy, composites, invasive species, lignin

INTRODUCTION

In the last century, most of energy and chemicals produced by industry were based on fossil fuels. The threat of depletion of fossil fuels and the environmental concerns related to their use at large scale has boosted the search for other sources and production processes. Lignocellulosic biomass is a renewable and ubiquitous feedstock with high potential for the production of biofuels and value-added chemicals. In particular, its high content in polysaccharides, allows to obtain from it, products so diverse as cellulose, hemicelluloses, furane derivatives, bioethanol, among others. One of its constituents, lignin, whose content can be as high as 35%, is often treated as a waste product, despite its high potential for numerous applications. As example, the black liquor obtained from the kraft cooking (in the pulp and paper industry), containing a great amount of lignin, is only heated to produce energy and no other valorization is given for it. Lignin, due to their rich aromatic structure, can be considered as a source of many derived products. By depolymerization, compounds such as vanillin, acetovanillin or syringaldehyde can be obtained. Lignin can still be functionalized and/or used as a component to produce composite structures. Additionally, lignin possesses interesting properties including antioxidant and antimicrobial properties, UV-protection ability, good thermal stability and

hydrophobicity. This work aims to isolate lignins from the wood of an invasive tree, and obtain new lignin-based products, thus valorizing, simultaneously, the wood of an invasive species and the lignin extracted from that wood. Lignins were isolated from *Acacia dealbata* wood using an ionic liquid and a deep eutectic solvent as “green” solvents and compared to kraft lignins, used as references. Their performance in the production of composites with polybutylene adipate terephthalate (PBAT), namely their impact on the mechanical properties of the PBAT-based material was studied.

EXPERIMENTAL

Materials and chemicals

Branches of *Acacia dealbata* collected in the central region of Portugal were used. These were cut into smaller fractions, ground and sieved to obtain a particle size of 0.25–0.84 mm. The chemicals required for the treatment of *Acacia* were [BMIM]MeSO₄, choline chloride and imidazole. These had a least 99% of purity and were used without any further purification. Methanol was also used in the washing steps.

The deep eutectic solvent based on choline chloride and imidazole was prepared by mixing the choline chloride (ChCl) and imidazole in a molar ratio of 3:7, at 100 °C, for 60 min, under magnetic stirring, as reported previously [1].

Two lignins were used as references for comparison purposes: one was a commercial lignin purchased from Sigma Aldrich; another was obtained from the black liquor of *Acacia dealbata* cooking (cooking conditions: 28% sulfidity, 20% active alkali charge, 160 °C, 1 hour). This lignin was precipitated by the addition of sulfuric acid to the liquor until pH 5.0. The precipitated lignin was filtered, washed with abundant amount of water, filtered again and dried at 60 °C.

Lignin extraction and isolation by Acacia dealbata wood treatments with IL and DES

The [BMIM]MeSO₄ and ChCl:imidazole were used for the *Acacia dealbata* treatments following the procedures developed in a previous study in which the conditions to extract lignin from wood were studied [1]. The extraction should conduct to a high lignin removal with minimal cellulose losses of the woody material.

Briefly, the pretreatment with [BMIM]MeSO₄ + H₂O was carried out by mixing 1 g (dry weight basis) of wood sawdust (0.25–0.84 mm), 2 g of distilled water and 8 g of IL in a Teflon lined stainless steel mini-reactor. Then, the reactor was placed in an oven kept at a specific temperature for a specific time. 140 °C for 24 h, 120 °C for 24 h and 140 °C for 8 h were the conditions selected for the IL treatment. The pretreatment with DES was carried out in a similar way, in which 1 g of the wood sawdust was mixed with 10 g of DES (DES/wood ratio of 10). For this treatment, the conditions used were 180 °C for 24 h, 160 °C for 24 h and 180 °C for 8 h. After the pretreatment, the treated sample was cooled and filtered under vacuum. The resulting solid (cellulose-rich material) was washed with methanol and an abundant amount of water, and dried, and the lignin-containing filtrate was collected for the further recover of lignin, as follows.

The methanol present in the filtrate was evaporated and water was added at a 10/1 (v/v) ratio to precipitate the material. The precipitated material (“lignin”) was filtered, washed with water and dried at 105 °C, for at least 4 h. The yield of lignin precipitation was calculated, as follows in equation 1.

$$\text{Precipitation yield (\%)} = \frac{m_{pm}}{m_{dl}} \times 100 \quad (\text{Eq. 1})$$

Here, m_{pm} is the mass of precipitated material (“lignin”), and m_{dl} is the mass of dissolved lignin during the IL or DES treatment. The mass of dissolved lignin is calculated by equation 2:

$$m_{dl} = m_{\text{lignin, original wood}} - m_{\text{lignin, cellulose-rich material}} \quad (\text{Eq. 2})$$

Here, m_{lignin} (original wood) is calculated by multiplying the lignin percentage in the raw material (*Acacia dealbata* wood) by the mass of wood used in treatment (dry basis); m_{lignin} (cellulose-rich material) is obtained by multiplying the lignin percentage in the cellulose-rich material by the mass of cellulose-rich material obtained (dry basis).

The chemical composition of the *Acacia dealbata* raw material and cellulose-rich materials obtained after the IL and DES treatments is summarized in Table 1 [1].

Table 1 - Composition of *Acacia dealbata* wood after pretreatments with [BMIM]MeSO₄ + H₂O and ChCl:imidazole

	Cellulose rich-material						
	T (°C)	t (h)	Dissolution yield (%)	Lignin (%)	Cellulose (%)	Xylan (%)	Delignification yield (%)
<i>Acacia dealbata</i>	---	---	---	20.2	47.4	18.8	
[BMIM] MeSO ₄ + H ₂ O	120	24	56.9 ± 0.8	6.0 ± 0.1	92.9 ± 1.7	3.7 ± 0.0	87.1 ± 0.5
[BMIM] MeSO ₄ + H ₂ O	140	8	59.9 ± 0.3	5.3 ± 0.0	90.5 ± 1.0	3.9 ± 0.6	89.4 ± 0.3
[BMIM] MeSO ₄ + H ₂ O	140	24	64.4 ± 0.0	4.3 ± 0.1	92.3 ± 1.2	2.3 ± 0.0	92.4 ± 0.2
ChCl:Imidazole	160	24	46.7 ± 0.3	7.0 ± 0.0	80.9 ± 1.2	14.7 ± 0.8	81.5 ± 0.0
ChCl:Imidazole	180	8	51.0 ± 0.9	5.6 ± 0.1	85.2 ± 0.5	9.3 ± 0.3	86.3 ± 0.5
ChCl:Imidazole	180	24	54.0 ± 0.2	4.3 ± 0.1	89.0 ± 1.9	9.4 ± 0.4	90.2 ± 0.2

Characterization of the lignins

The lignins were characterized for their purity by measuring the total lignin content and the carbohydrates' content. Total lignin comprises the Klason lignin (insoluble fraction) and the acid-soluble lignin that are obtained after a two-step hydrolysis with sulfuric acid. The carbohydrates' content was determined by high-performance liquid chromatography in a Knauer instrument equipped with a refractive index detector and a Rezex ROA-organic acid column from Phenomenex.

The lignin samples were also analyzed by Fourier transform infrared (FTIR) spectroscopy. FTIR spectra were obtained on a PerkinElmer spectrometer coupled with an attenuated total reflectance (ATR) unit, using 128 scans and a resolution of 4 cm⁻¹, in the range of 500–4000 cm⁻¹.

Vis-UV reflectance spectra were obtained in a Jasco V-560 spectrophotometer with a Jasco ISV-469 integrating sphere. Spectra in the reflectance mode were converted into k/s Kubelka-Munk units. Spectra were run from 900 to 190 nm with a scanning speed of 100 nm/min and a bandwidth of 5 nm. Lignin samples were previously diluted with MgO, due to their intense brown color, in order to have k/s units in an appropriate range (ca. 1.5-2), and the spectra were registered using MgO as background reference.

Thermal analysis was carried out using a SDT Q600 thermal analyzer from TA instruments. The samples were heated under a nitrogen flow (100 mL/min) from room temperature up to 900 °C, at a rate of 10 °C/min. Derivative curves were also obtained, which enabled to assess the peaks of maximum thermal degradation rate. As well, char residue was quantified and compared for the different lignin samples. Activation energies for the main step of lignin degradation were also calculated, based on the approach described by equation 3.

$$\ln \left(\ln \left(\frac{1}{y} \right) \right) = - \left(\frac{E_a}{R} \right) \times \left(\frac{1}{T} \right) \quad (\text{Eq. 3})$$

Here, E_a is the activation energy (kJ mol^{-1}), R is the universal gas constant ($R = 8.314 \text{ J mol}^{-1}\text{K}^{-1}$), T is the temperature in Kelvin, and y is the fraction of undecomposed material remaining at a temperature T , and defined as $(m_T - m_\infty) / (m_0 - m_\infty)$. Here, m_T is the sample mass at the temperature T , m_0 and m_∞ are the sample masses at the beginning and at the end of the thermal decomposition, respectively. The activation energy is obtained from the slope of the linear fit of $\ln(\ln(1/y))$ versus $1/T$ in a range of temperature.

RESULTS AND DISCUSSION

Lignin extraction from Acacia dealbata wood using green solvents and characterization of the isolated lignins

It was found that delignification (lignin removal from the original wood material) larger than 90% could be obtained under optimized conditions (Table 1). A high and selective lignin removal are obviously preferred, aiming the further recover of a high-purity lignin from the dissolved fraction. The conditions specified in Table 1, with lignin removals not lower than 80% were, therefore, the chosen ones for the present study focused on the lignin extraction using green solvents, and on their isolation and utilization.

Following the *Acacia dealbata* wood treatments with $[\text{BMIM}]\text{MeSO}_4 + \text{H}_2\text{O}$ and $\text{ChCl}:\text{imidazole}$, lignin was obtained from the dissolved wood fractions (filtrates). The addition of water as anti-solvent was found to be enough for the lignin precipitation. The results of the yield, lignin content, and polysaccharides' content of the isolated lignins are shown in Table 2.

Table 2 – Yield and chemical composition of the lignins precipitated from the pretreatments of *Acacia dealbata* wood with $[\text{BMIM}]\text{MeSO}_4 + \text{H}_2\text{O}$ and $\text{ChCl}:\text{imidazole}$

	T (°C)	t (h)	Precipitated lignins					Polysaccharides (%) [*]
			Lignin yield (%)	Klason lignin (%)	Soluble lignin (%)	Total lignin (%)	Klason lignin/total lignin ratio	
$[\text{BMIM}]\text{MeSO}_4 + \text{H}_2\text{O}$	120	24	67.8 ± 1.5	86.7 ± 3.0	1.6 ± 0.1	88.3 ± 3.1	0.98	0.89 ± 0.17
$[\text{BMIM}]\text{MeSO}_4 + \text{H}_2\text{O}$	140	8	80.9 ± 1.3	81.1 ± 0.3	2.0 ± 0.1	83.2 ± 0.2	0.97	0.59 ± 0.03
$[\text{BMIM}]\text{MeSO}_4 + \text{H}_2\text{O}$	140	24	112.4 ± 1.4	90.8 ± 1.5	1.8 ± 0.04	92.6 ± 1.4	0.98	0.30 ± 0.05
$\text{ChCl}:\text{imidazole}$	160	24	75.7 ± 2.7	83.6 ± 0.4	9.1 ± 0.6	92.7 ± 0.2	0.90	0.61 ± 0.03
$\text{ChCl}:\text{imidazole}$	180	8	68.2 ± 1.5	79.6 ± 1.8	11.4 ± 0.2	90.9 ± 2.0	0.88	0.33 ± 0.08
$\text{ChCl}:\text{imidazole}$	180	24	98.4 ± 0.8	64.7 ± 1.3	20.5 ± 0.9	85.2 ± 2.2	0.76	0.17 ± 0.05

^{*}content of cellulose + xylan (from the HPLC determination of glucose and xylose in the hydrolysis filtrate)

The yield of precipitated lignin material was determined by dividing the amount of precipitated material by the amount of dissolved lignin (equations 1 and 2). For this calculation, it was assumed that the isolated lignin material comprises 100% of lignin. However, minor amounts of other components previously dissolved during the treatment with DES and ionic liquid could also be present in the precipitated lignin, e.g., polysaccharides. The possibility of the presence of ionic liquid and eutectic solvent residues in the precipitated lignin cannot be discarded as well, although no evidence was found for that. Note, as well, that errors involved in the lignin determination will also affect the accuracy of the dissolved lignin calculation (equation 2), and therefore, the lignin yield (equation 1).

Even so, the results indicate good yields of lignin recover, approaching 100% for the more severe conditions of extraction (140 °C; 24 h for IL + H₂O and 180 °C; 24 h for the DES). For less severe conditions, lignin yields were in the range of 68-81%, which can also be considered acceptable values for a practical application of the method of lignin extraction and precipitation. A yield higher than 100% must certainly reveal some contamination of the isolated “lignin”.

The lignin content in the precipitated lignins was determined, in order to assess the purity of the isolated lignins. The total lignin content of the precipitated materials varied between 83% and 93%, indicating good purities of the isolated lignins. In particular, for the more severe conditions of wood extraction with IL + H₂O (140 °C; 24 h), the lignin content of the precipitated material was 93%, lower purities having been obtained under the other conditions. On the other hand, for the extractions of lignin with DES, the highest lignin content (93%) was obtained for the extraction at lower temperature (160 °C; 24 h). With regards to the Klason lignin and soluble lignin, the trends were the same of the total lignin. The Klason lignin values were lower for the lignins isolated after the DES treatments *versus* those obtained after the IL + H₂O treatments, and the soluble lignin was significantly higher with the DES treatments. The Klason lignin/total lignin ratio values shown in Table 2 confirm a higher relative amount of Klason lignin in the lignins obtained with IL + H₂O (ratios of 0.97-0.98 versus 0.76-0.90). The lowest Klason lignin/total lignin ratio was obtained for the lignin following DES extraction at 180 °C for 24 h (0.76, corresponding to 65% of Klason lignin, 20.5% of soluble lignin and 85% of total lignin). The differences in the relative contents of Klason lignin and acid-soluble lignin when comparing lignins obtained from the IL treatments with those from the DES treatments indicate different structural features and degree of polymerization of the isolated lignins.

Lignin has been isolated from other lignin sources after DES extractions followed by precipitation, and its purity evaluated as well. Lignin purities in the range of 93-98% have been claimed [2-4]. For instance, using the same DES of the present study (ChCl:imidazole) and precipitation with water yielded a lignin from poplar wood with a purity of 94% [3]. The present results are not far from those previously published, showing that the conditions here applied for the delignification and lignin precipitation of an invasive tree species were as effective as revealed in other studies.

To assess the possible contamination of lignin by polysaccharides that have also been dissolved during the wood treatment with the green solvents tested, the content in carbohydrates was determined. Only glucose and xylose were detected by HPLC. Additionally, the total polysaccharides were of less than 1% (Table 2). Therefore, the isolated lignins are almost free of contamination by polysaccharides, confirming their high purity evidenced by the lignin determinations. These results show the high selectivity of the applied method of lignin precipitation for the systems under study.

The Vis-UV spectra (*k/s*) of the lignins are shown in Figure 1 and the data summarized in Table 3.

From the data in Table 3, it is clear that the wavelength corresponding to the *k/s* maximum varies with the lignin type. For the lignins obtained with IL, the *k/s* maximum was at 480-530 nm. For the lignins obtained with DES, *k/s* maximum was at 390-400 nm. Additionally, all IL- and DES-lignins showed higher absorption in the visible region than the kraft lignin from Sigma (used as reference). Compared with *Acacia* Kraft lignin (used as reference of the same raw material), the IL-lignins showed an absorption maximum in a similar range and a closer Vis-UV spectrum (Figure 1).

The lignin samples were analysed by thermogravimetry. The thermograms and the corresponding derivative curves are shown in Figure 2. The data obtained from the thermal analysis are summarized in Table 4. Thermogravimetric plots showed mainly two steps of weight loss, the initial one due to the release of adsorbed water, and the second one, starting at about 200 °C, due to the thermal degradation of the lignin samples. Lignin thermal degradation started with the degradation of the lignin lateral chains followed by the cleavage of internal linkages of lignin with the release of volatile compounds [5]. From the derivative curves, it was observed that the IL-lignins present higher thermal stability than the Sigma Kraft lignin and the *Acacia* Kraft lignin (as measured by the temperature of maximum thermal degradation rate (T_{max}) in the derivative curve). For the IL-lignins, it was also observed an increase in thermal stability with the increase in severity of treatment conditions (IL 140°C/24h >> IL 140°C/8h > IL 120°C/24h). The DES-lignin samples showed lower thermal stability than the IL-lignin samples. Here, more severe conditions of lignin extraction also conducted to better thermal stability of

the recovered lignins (DES 180°C/24h >> DES 180°C/8h > DES 160°C/24h).

Char residue of the IL and DES-lignins was in the range of 43-50%. The largest char residue was obtained for the lignin from IL 140°C/24h (ca. 50%). As for the activation energy of the main step of lignin thermal degradation (including the T_{max}), this was higher for the lignins obtained from ionic liquid extraction. The IL-lignins showed similar activation energies of that of *Acacia* kraft lignin.

FTIR spectra showed bands that can be ascribed to the presence of lignin. Among the series of spectra of lignins obtained from extraction with ionic liquid, the spectra were fairly similar. The spectra of lignins obtained from extraction with eutectic solvent were also similar to each other. However, when comparing lignins from IL extraction with those from DES extraction, some differences were noted. For instance, the band at ca. 1700 cm^{-1} present in the spectra of IL-lignins is almost absent in the spectra of DES-lignins. Differences were also noted in the region of 600-900 cm^{-1} . The main bands were observed at 1594-1598, 1510, 1455, 1423, 1208, 1108 (IL-lignin), 1082 (DES-lignin) and 1030 cm^{-1} . These bands reflect the C-H vibrations in aromatic rings (out-of-plane and in-plane), the C-O and O-H stretching and bending modes. The FTIR spectra certainly deserve a deeper analysis that is out of the scope of the present proceeding.

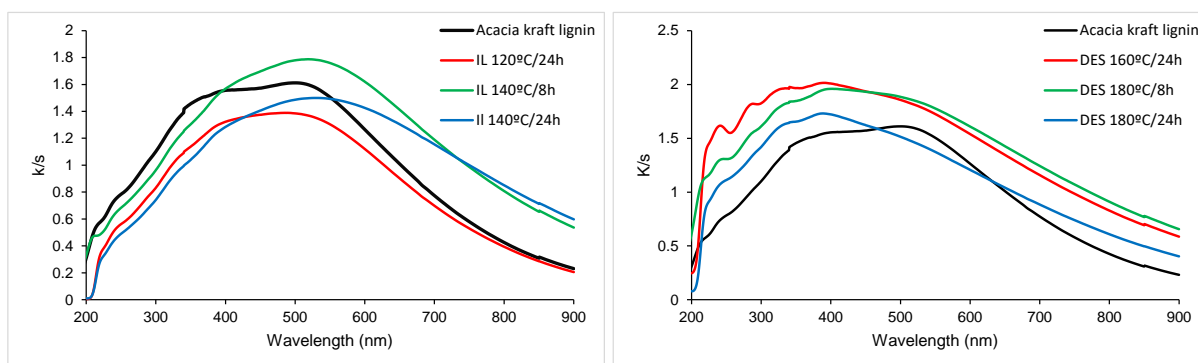


Figure 1: Vis-UV (k/s) spectra of IL- and DES-lignins compared with *Acacia* kraft lignin.

Table 3 – Vis-UV data for lignin samples

Sample	<i>k/s</i> maximum Wavelength (nm)
Sigma Kraft lignin	357
<i>Acacia</i> Kraft lignin	501
IL 120°C/24h	484
IL 140°C/8h	519
IL 140°C/24h	528
DES 160°C/24h	391
DES 180°C/8h	402
DES 180°C/24h	389

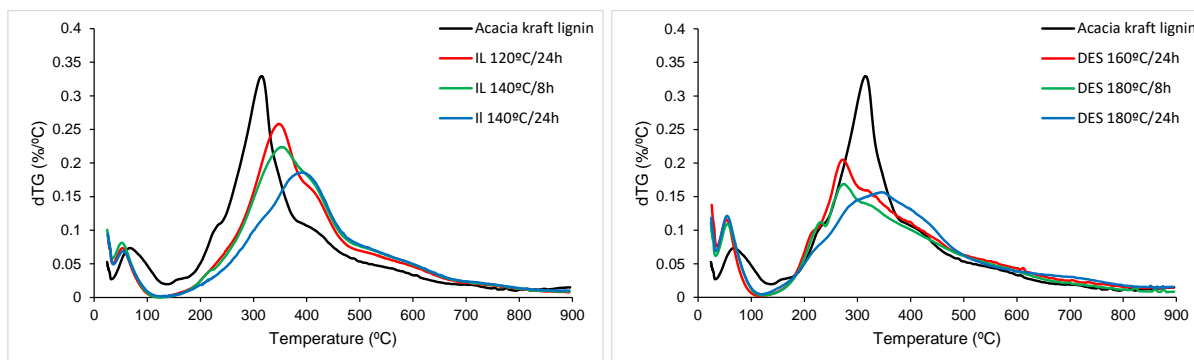


Figure 2: Thermogram derivative curves of IL- and DES-lignins compared with *Acacia* kraft lignin.

Table 4 – Thermogravimetry data of the recovered lignins

Sample	T _{max} (°C)	Char residue (%)	Actvation energy/main step (KJ/mol)*
Sigma Kraft lignin	322	45.7	36.5
<i>Acacia</i> Kraft lignin	315	41.0	41.0
IL 120°C/24h	347	43.5	43.4
IL 140°C/8h	354	43.7	40.5
IL 140°C/24h	391	49.7	39.3
DES 160°C/24h	271	43.4	31.9
DES 180°C/8h	274	49.3	29.0
DES 180°C/24h	345	45.5	26.8

*Interpolation regions were 270-400°C for IL 120°C/24h and IL 140°C/8h; 310-440°C for IL 140°C/24h; 230-330°C for DES 160°C/24h and DES 180°C/8h; 240-400°C for DES 180°C/24h; 250-380°C for Sigma kraft lignin and 250-350 °C for *Acacia* Kraft lignin.

Composites of lignin and PBAT

Composites were produced with PBAT and 10, 20 and 30% of lignin (w/w). Kraft lignin from Sigma, *Acacia* Kraft lignin and DES-lignins (160°C/24h and 180°C/24h) were tested. PBAT has a low Young's modulus, a moderate tensile strength and a very high elongation at break. Preliminary results showed that the incorporation of lignin increases the tensile strength and Young's modulus, while the elongation at break is reduced. Therefore, lignin can be proposed as a promising material aiming at the improvement of stiffness of PBAT-based materials.

CONCLUSIONS

It was possible to obtain yields of lignin recover approaching 100% for the more severe conditions of lignin extraction with IL + H₂O (140 °C; 24 h) and DES (180 °C; 24 h). Additionally, the isolated lignins were reasonably pure, with total lignin (Klason lignin plus acid-soluble lignin) values between 83 and 93% and the presence of only minor amounts of polysaccharides (less than 1%). When used in the production of composites with polybutylene adipate terephthalate (PBAT), a promising polymer for plastic production, lignins were found to improve stiffness of the resultant materials.

ACKNOWLEDGMENTS

Authors thank support from the Strategic Research Centre Project, UIDB/00102/2020, funded by Fundação para a Ciência e Tecnologia.

REFERENCES

1. Almeida, R. O., Moreira, A., Moreira, D., Pina, M. E., Carvalho, M. G. V. S., Rasteiro, M. G., & Gamelas, J. A. F. (2022). High-performance delignification of invasive tree species wood with ionic liquid and deep eutectic solvent for the production of cellulose-based polyelectrolytes. *RSC Advances*, 12(7), 3979–3989. <https://doi.org/10.1039/d1ra08410k>
2. Chen, Y., Zhang, L., Yu, J., Lu, Y., Jiang, B., Fan, Y., & Wang, Z. (2019). High-purity lignin isolated from poplar wood meal through dissolving treatment with deep eutectic solvents. *Royal Society Open Science*, 6(1), 181757. <https://doi.org/10.1098/rsos.181757>
3. Li, H., Li, X., You, T., Li, D., Nawaz, H., Zhang, X., & Xu Feng. (2021). Insights into alkaline choline chloride-based deep eutectic solvents pretreatment for *Populus deltoides*: Lignin structural features and modification mechanism. *International Journal of Biological Macromolecules*, 193, 319–327. <https://doi.org/10.1016/j.ijbiomac.2021.10.134>
4. Su, Y., Huang, C., Lai, C., & Yong, Q. (2021). Green solvent pretreatment for enhanced production of sugars and antioxidative lignin from poplar. *Bioresource Technology*, 321, 124471. <https://doi.org/10.1016/j.biortech.2020.124471>
5. Lyu, G., Wu, Q., Li, T., Jiang, W., Ji, X., & Yang, G. (2019). Thermochemical properties of lignin extracted from willow by deep eutectic solvents (DES). *Cellulose*, 26(15), 8501–8511. <https://doi.org/10.1007/s10570-019-02489-8>