Decoloration of organosolv lignin from sugarcane bagasse by the oxidation process with sodium hypochlorite

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Abstract

Among lignin applications, the use of lignin as a natural or organic pigment similar to plant pigments and flower pigments has been considered for potential use in industrial applications such as dyestuff, cosmetics, and plastic products. However, the physical appearance of lignin with dark or light brown color and black shade has been a drawback in terms of using the above application. In this work, the preparation of organosolv lignin extracted from sugarcane bagasse (SB) followed by a study of lignin modification by an oxidative process using sodium hypochlorite (NaOCl) was carried out with the aim of decolorization and the increase of lignin properties. The results indicated that a bleaching agent with 10% NaOCl was the most effective for lignin decoloration, as evidenced by the highest brightness of 68.29 L value with the maximum average color difference ($\Delta E = 37.1$) compared to unmodified conventional lignin. Moreover, all modified lignin by the NaOCl oxidative process presented higher UV protection and antioxidant activity correlated to higher OH content compared to conventional organosolv lignin. The research in this work expected to decolorize lignin while still having the anti-UV and antioxidant properties based on using a commercial NaOCl oxidizing agent.

Keywords: *Decoloration, Organosolv lignin, Sugarcane bagasse, Oxidation, Sodium hypochlorite*

1. Introduction

Agricultural residue from sugarcane, rice, corn, and oil palm has been utilized as feedstocks for biomass conversion, particularly sugarcane bagasse obtained from the sugarcane industry, which is an important crop in Thailand and across the world. Although sugarcane waste products are often utilized as a fuel source in furnaces for energy benefits, they can also be used as alternative feedstocks in biorefinery platforms, resulting in an increase in income and market channels for the traditional sugar sector (Seabra, Tao et al., 2010). Lignocellulosic biomass is composed mostly of three important components: hemicellulose (23–32%), lignin (15–25%), and cellulose (38–50%) (Kumar, Biswas et al., 2020). Lignin is the most prevalent aromatic polymer in nature, accounting for 18-35 wt% of lignocellulosic biomass depending on the species group (Wen, Sun et al., 2013). Lignin is found on the outer surface of the cell wall in the middle lamella and is covalently bonded to hemicelluloses. The corresponding aromatic portions of these alcohols are known as p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units within the lignin structure.

These units are connected by either ether bonds (C-O-C) like β-O-4, α-O-4, and 4-O-5 links, or carbon-carbon (C-C) bonds like β-5, β-1, 5-5, and β-β linkages (Kumar, Biswas et al., 2020).

Substances made from carbon such as plant, flower, stem, and tissue culture have traditionally been used to make organic colors. In the same way as plant and flower pigments are used in dyestuff, cosmetics, and polymers, lignin is a promising biomass structure that has the potential to be used as a natural or organic pigment in industrial applications. The chromophoric and leucochromophoric groups, which make up the internal structure of lignin, are responsible for its color. The following five chromophores are generally thought to be possible: (1) carbon–carbon double bonds conjugated with the aromatic ring; (2) quinone methides and quinones; (3) chalcone structures; (4) free radicals; (5) metal complexes with catechol structures (Wang, Deng et al., 2016). Furthermore, lighter or whiter-colored lignin results from the blockage of free phenolic hydroxyl groups by oxidation with air, molecular oxygen, hydrogen peroxide, and sodium hypochlorite. Sodium hypochlorite dissociates in water to form hypochlorous acid (HOCl) and hypochlorite ions (OCl^-). The active oxidizing species is hypochlorous acid (HOCl) (Fukuzaki, 2006), which can react with lignin through oxidative reactions. The pH of hypochlorite bleach ranges from 11 to 13. It usually comprises 3- 10% NaOCl and 0.01-0.05% NaOH to prevent the bleach from breaking down into inert sodium ions [9]. The quantity of free chlorine (Cl_2) released when 100 milliliters of solution are acidified is known as "available chlorine," and it is commonly used to indicate the strength of an NaOCl solution (Mayer and Miskelly, 2023).

In the present work, the preparation of organosolv lignin extracted from sugarcane bagasse (SB) was proposed followed by a study of lignin modification by the oxidative process using sodium hypochlorite (NaOCl) with the aim of color change and/or the decolorization of lignin. The efficiency of lignin modification was considered in terms of color change, total OH content, and interesting lignin properties (anti-UV and antioxidant). The effect of NaOCl concentration under different conditions (2%, 4%, 8%, and 10%) was studied in comparison with commercial iron oxide and conventional organosolv lignin. This work provides a fundamental analysis of lignin modification with lighter colors as well as the improvement of UV-protection and antioxidant properties, leading to the possibility of potential use in further industrial applications.

2. Objectives

1. There can be a prototype of decolorized lignin and the decolorized lignin can also be used.
2. Lignin that has been stripped of its original color retains its functional properties.

3. Materials and Methods

3.1 Materials

Sulfuric acid and ethanol were produced by Fisher Scientific Co. L.L.C. Sodium hypochlorite was produced by Apex Chemicals Co., Ltd. All the chemical reagents were of analytical grade and were used without further purification. The sugarcane bagasse (SB) was obtained from a sugar mill factory.

3.2 Preparation of organosolv lignin by ethanol

3.2.1 Biomass preparation

The raw SB was obtained from a sugar mill factory. It was cut and crushed into tiny bits using a pin mill model 30-BV with a size of 1-4 mm. The moisture content was then decreased by drying it at 70 °C.

3.2.2 Organosolv pretreatment

The SB sample (500 g) was prepared using 4 L of a 70:30 v/v ethanol and water mixed solvent, with 1% w/w H_2SO_4 added based on the weight of biomass loading. This organosolv process was operated in a 7.5 L high-pressure reactor that was heated using an electric jacket and a thermocouple to gauge internal temperature. The reaction was conducted at 175 °C for 60 minutes while being stirred at 300 rpm 0 bar (Agnihotri, Johnsen et al., 2015). The reaction was stopped immediately after the organosolv procedure by quenching on ice for ten minutes. The solid portion of the pretreatment sample was separated from the liquid phase using a centrifuge and filter bag. Afterward, the liquid fraction of the aqueous-organic combination was recovered, and a third of the amount of water was added to precipitate the resulting organosolv lignin. The lignin was recovered by filtration with a 110 mm paper filter (FiltraTECH, France). The lignin samples were dried for a few days at room temperature. The lignin yield used for this study was calculated from the combined mass of the lignins isolated from the aqueous and organic fractions (Astner, Young et al., 2015)(Equation 1).

$$\%\, Lignin\ yield = \left(\frac{Lignin\ after\ Pretreatment\ (g)}{Klason\ lignin\ in\ biomass\ (g)}\right) x\ 100. \qquad \text{(Equation 1)}$$

3.3 Klason Lignin

The National Renewable Energy Laboratory (NREL) offered a laboratory analytical approach that allowed for the determination of the composition of lignin recovered from the organic phase based on the Klason lignin content. Lignin underwent treatment with 72% v/v sulfuric acid at ambient temperature for a duration of 2.0 hours, after which it was diluted to 3% sulfuric acid concentration and subjected to autoclaving at 121°C for 1 hour. Subsequently, the treated lignin was filtered using a tared crucible, washed, dried, and then weighed. The weight represents the Klason lignin (AIL) value. The liquid obtained from the experiment was then measured with a UV spectrometer (model UV-5100PC, SN: AE2103080) at a wavelength of 514 nanometers to obtain the value of Acid-soluble lignin (ASL) and was then injected with HPLC to measure the sugar contaminants in the lignin.

3.4 Decoloration of lignin by the oxidation process using sodium hypochlorite

The reactions contained 1 g of lignin with 100 mL of NaOCl solution (2, 4, 8, and 10 %v/v) in a flask and were stirred for 0.5 h at 70 °C. The pH was adjusted to 4 by sulfuric acid. Then, the lignin was precipitated by centrifuging at 9000 rpm and then dried at room temperature.

3.5 Characterization of decolorized lignin samples

3.5.1 Color test

In the color measurement test, the color of the decolorized lignin was measured using a color tester model PRECISE COLOR READER OPERATION CS-220. When the lignin was reduced in color, 0.5 lignin was placed into the machine in grams, then measured with a colorimeter. The coordinates L* brightness a* and b*, represented as "-a*" (green), "+a*" (red), "b*" (blue), and "+b*" (yellow), respectively, which gave the values of CIE L*a*b* system in units. The color difference (⊗E) was calculated that follows (Wu, Yang et al., 2023):

$$\Delta E = \sqrt{(L_0 * - L_1 *)^2 + (a_0 * - a_1 *)^2 + (b_0 * - b_1 *)^2} \qquad \text{(Equation 2)}$$

where$(L_0 * - L_1 *), (a_0 * - a_1 *), and\ (b_0 * - b_1 *)$ are differences between each color value of the standard color lignin and oxidized lignin. Four measurements taken for each lignin are reported.

3.5.2 UV protection test (SPF and UVA test) UV absorption ability

Samples of lignin powder were transferred to a quartz cuvette for UV transmittance testing and then dissolved in 70% v/v ethanol to a final concentration of 5%w/v. Using a cuvette containing 70% v/v ethanol, the UV spectrometer (model UV-5100PC, SN: AE2103080) was reset to zero. UVB (290-320 nm) and UVA (320-400 nm) wavelength ranges comprised the 290-400 nm UV transmittance test periods. For each sample, three spots were scanned, and the average transmittance value was determined. This equation was used to calculate the SPF values:

$$SPF\ (UVA) = \sum_{320}^{400} \square\, E_\lambda S_\lambda / \sum_{3200}^{400} \square\, E_\lambda S_\lambda T_\lambda \qquad \text{(Equation 3)}$$

$$SPF\ (UVB) = \sum_{290}^{320} \square\, E_\lambda S_\lambda / \sum_{290}^{320} \square\, E_\lambda S_\lambda T_\lambda \qquad \text{(Equation 4)}$$

where E_λ is the CIE erythemal spectral efficacy, S_λ indicates sun spectrum irradiance, and T_λ denotes sample spectrum transmittance.

3.5.3 Antioxidant activity

To measure the antioxidant activity of the lignin samples in vitro, 2,2-diphenyl-1-picrylhydrazil (DPPH) (Dizhbite, Telysheva et al., 2004) was used. After mixing one milliliter of sample solution (10–200 g/mL) with 2.5 milliliters of DPPH methanolic solution (100 g/mL), the combination was incubated for 40 minutes at 25°C in the dark. A microplate was used to test the absorbance of liquids. After 40 minutes of incubation (Abs sample), the reader (SPECTROstar® Nano) was calibrated at 517 nm. With relative variances of less than 5%, the experiment was carried out in triplicate. The value was calculated using the equation below.

$$AA\% = 100 - \left[\frac{(Abs_{Sample} - Abs_{blank}) \times 100}{Abs_{control}}\right] \qquad \text{(Equation 5)}$$

where $\%\,Antioxidant\ activity\ (AA\%\ )$, Abs_{Sample} is the sample after 40 minutes of incubation, Abs_{blank}indicates the absorbance of the sample without the DPPH solution, and $Abs_{control}$ notes only the DPPH solution without a sample.

4. Result and Discussions

4.1 Preparation of organosolv lignin

The lignin was extracted from the organosolv process containing the experimental condition with 500 grams SB by using 70% ethanol at 175 °C. Organosolv lignin was recovered with a yield of 53.44% w/w **(Table 1)**. Moreover, the extracted organosolv lignin as conventional lignin showed an excellent purity of 95.22% with less contamination of sugar, suggesting the efficiency of the organosolv process in lignin extraction. During the process, lignin undergoes delignification by organic solvents and becomes dissolved in the mixture, while some hemicellulose is isolated. Mechanistically, the primary breakdown occurs in lignin-carbohydrate and α-O-4' ether linkages, with some β-O-4' potentially cleaved to yield lignin oligomers. The separation of lignin is achievable by reducing the pH of the mixture; once precipitated, the lignin undergoes filtration and subsequent drying. This method stands out as one of the most efficient means of lignin valorization since it can effectively preserve the structure of lignin akin to native lignin. Furthermore, organosolv represents a cleaner and less wasteful process. Nonetheless, its limited commercial use as a pulping method is attributed to concerns regarding low pulp quality and equipment corrosion (Figueiredo, Lintinen et al., 2018; Ekielski, Żelaziński et al., 2021).

Table 1: Chemical composition of extracted organosolv lignin as conventional lignin

Specification (%w/w)	SB
Total lignin	96.38 ± 1.14
Klason lignin (AIL)	95.22 ± 1.06
Acid-soluble lignin (ASL)	1.16 ± 0.08
Total sugar	2.43 ± 0.27
Glucose	0.07 ± 0.64
Xylose	0.91 ± 0.14
Arabinose	0.75 ± 0.17
Ash	1.19 ± 0.17

%Lignin yield	53.44 ± 0.54 %

4.2 Color measurement of modified lignin

Oxidation with sodium hypochlorite led to the decolorization of extracted lignin, as seen in **Figure 1.** The untreated lignin sample and iron oxide used as a commercial colorant in various industries such as the cosmetics industry, had black, dark brown, and deep yellow colors, while the oxidized lignin demonstrated a very light color. Increasing hypochlorite concentration led to a lighter color of lignin, especially using an oxidative process with 10% hypochlorite with an almost white lignin color. In addition, the color level of lignin samples was evaluated by their brightness and L*a*b* values**.** The chromaticity coordinates are represented by the L* coordinate, representing the psychometric index of lightness from 0 (black, darkness) to 100 (white, brightness). The coordinates a* and b*, are represented as "-a*" (green), "+a*" (red), "b*" (blue), and "+b*" (yellow), respectively (Wen, Sun et al., 2013). According to the results in **Figure 2**, it was shown that 10% NaOCl was the most effective in decolorizing lignin, showing L*a*b values of 68.29, 1.53, and 20.53, respectively; 10 % NaOCl was the highest commercial concentration. Comparing the brightness and lightness of untreated and oxidized lignin, this indicates an improvement in the ⊗E of color change at 37.1 using 10 % NaOCl. The results indicated that the block of free phenolic hydroxyl groups of modified lignin by oxidation process leads to lighter or whitened lignin color (Wang, Deng et al., 2016). It can be seen that lignin was bleached using an oxidation process with NaOCl, which caused the lignin-containing aromatic rings to break down. This bleaching agent oxidizes, breaking down some of the chromophoric groups within the lignin, resulting in a brighter color. Previous research also indicates that bleach is frequently used. This is because it is strongly oxidized to make the pulp whiter and destroys the lignin structure that gives it color. This creates lignin, which makes it whiter. Changes in sodium hypochlorite concentration affect its physical characteristics (Aridi et al., 2017).

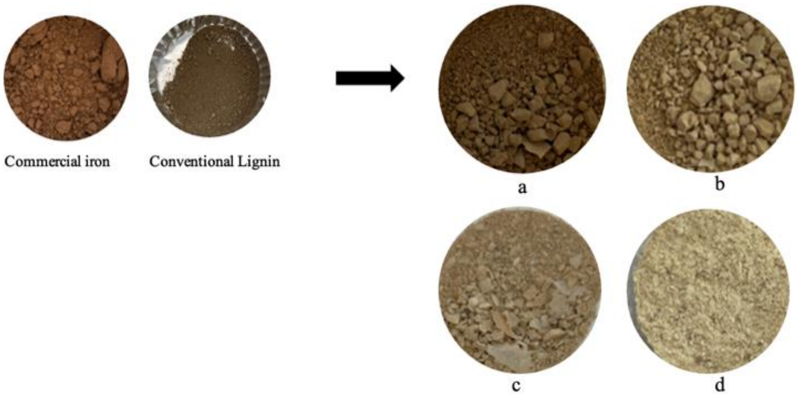

Figure 1: Oxidation of lignin by the NaOCl bleaching process using different concentrations of a) 2% NaOCl, b) 4% NaOCl, c) 8% NaOCl, d)10% NaOCl

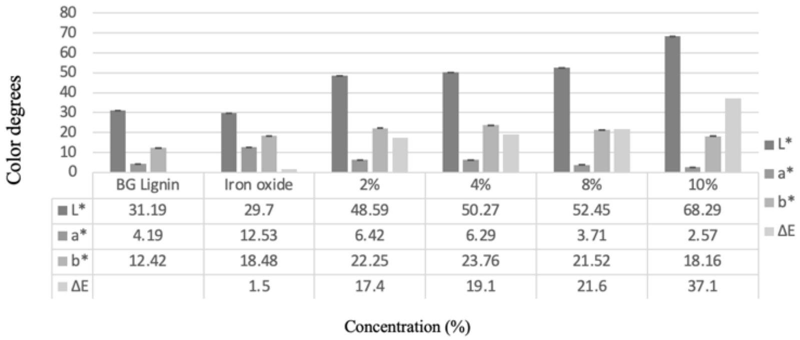

Figure 2: Color degrees of oxidized lignin, commercial iron oxide, and conventional organosolv lignin

4.3 Properties of modified lignin

Lignin oxidation by NaOCl represents a straightforward and effective approach for lignin decolorization. The UV absorbance of oxidized lignin was compared within the UVA (320–400 nm) and UVB (290–320 nm) ranges. At 2% NaOCl, the results provided the maximum UVA and UVB protection at 47.86 and 48.35, respectively **(Figure 3a)**. Moreover, the increase of NaOCl concentration in the oxidation process affected the decrease of UV protection in oxidized lignin. However, all oxidized lignin achieved the higher UV protection compared to the conventional lignin. It could be due to increases in phenolic, hydroxyl, auxochrome, and catechol units, as well as increasing UV absorption (Widsten, Tamminen et al., 2020). This result corresponded to an increase of OH content under the oxidative process of lignin compared to conventional lignin (**Figure 3C)**. The phenolic hydroxyl content of lignin suggests a strong correlation between phenolic hydroxyls and the efficacy of lignin sunscreen. Qian, Zhong et al. (2017) also emphasized the significance of phenolic hydroxyls in this context. However, one of the primary advantages of lignin over certain other commercial organic filters and other natural phenolic compounds, such as extracts, is its capability to block the entire spectrum of UV light (Widsten, Tamminen et al., 2020).

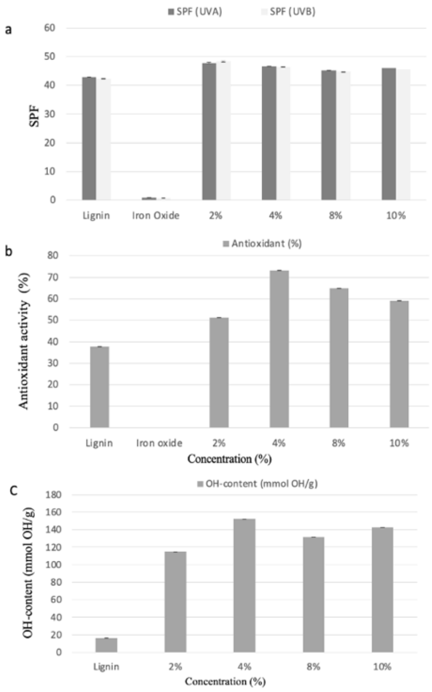

Figure 3: Properties of oxidized lignin, commercial iron oxide, and conventional organosolv lignin in terms of a) UV Protection, b) % antioxidant, and c) OH content

The antioxidant properties of the lignin samples were assessed using the DPPH scavenging analysis. The results showed that the oxidation of lignin using NaOCl increased free radical scavenging activity, as depicted in **Figure 3b**. The 4% NaOCl concentration exhibited the highest antioxidant activity at 73.16% compared to untreated lignin and commercial iron oxide at 37.66% and 0%, respectively. This result is consistent with the maximum lignin OH content determined by the stepwise titration of ASTM E222 for results treated with 4% sodium hypochlorite, which reached a peak of 152.30 mmol/g, as in **Figure 3c**. Generally, the antioxidant activity of lignin correlates positively with the presence of free phenolic hydroxyl groups. This outcome aligns with the total phenolic content findings of lignin. Generally, the antioxidant activity observed in the lignin samples in this study corresponds to their phenolic hydroxyl content. This correlation has been documented in related literature (Dizhbite, Telysheva et al., 2004). Its antioxidant capacity primarily stems from its capability to eliminate oxygen-containing groups and substitute oxidation via helix and carbonyl groups. Dizhbite, Telysheva et al. (2004) noted that lignin-containing S-units exhibit enhanced reducing power activity, consequently leading to improved antioxidant activity. Conversely, the antioxidant activity of lignin compounds demonstrates a negative correlation with the abundance of the aliphatic hydroxyl group (Ugartondo, Mitjans et al., 2008). The color, UV protection, antioxidant activity, and OH content properties based on oxidized lignin and conventional lignin are summarized in **Table 2.**

Table 2: Characterization of modified lignin, commercial iron oxide, and conventional organosolv lignin at different NaOCl concentrations

Sample			L*	a*	b*	ΔE	UVA	UVB	% AA	OH-content (mmol/g)
Commercial iron oxide			29.70	12.53	18.48	1.5	0.8269	0.7363	n/a	n/a
Conventional organosolv lignin			31.19	4.19	12.42	n/a	42.88	42.39	37.66	16.33
Modified lignin	% NaOCl concentration	2	48.59	6.42	22.25	17.4	47.86	17.4	51.22	114.79
		4	50.27	6.29	23.76	19.1	46.66	19.1	73.16	152.30
		8	52.45	3.71	21.52	21.6	45.29	21.6	64.80	131.747
		10	68.29	1.53	20.53	37.1	46.11	37.1	59.13	142.64

5. Conclusion

In this work, organosolv lignin was decolorized by oxidation treatment using NaOCl. The result was a brighter lignin color by increasing the NaOCl concentration due to breaking down some of the chromophoric groups within the lignin. In addition, the efficiency of oxidized lignin, in terms of UV protection and antioxidant activity, can be enhanced compared to commercial iron oxide and conventional lignin, resulting from an increase in total OH content after the oxidative process. The brighter color, higher UV protection, and antioxidant activity of modified lignin by NaOCl agent could potentially be applied in various industries, such as for dyestuff, cosmetics, and plastic products.

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7. References

Agnihotri, S., I. A. Johnsen, M. S. Bøe, K. Øyaas and S. Moe (2015). "Ethanol organosolv pretreatment of softwood (Picea abies) and sugarcane bagasse for biofuel and biorefinery applications." Wood Science and Technology **49**(5): 881-896.

Astner, A., T. Young and J. Bozell (2015). "Lignin yield maximization of mixed biorefinery feedstocks by organosolv fractionation using Taguchi Robust Product Design." Biomass and Bioenergy **73**: 209-206.

Dizhbite, T., G. Telysheva, V. Jurkjane and U. Viesturs (2004). "Characterization of the radical scavenging activity of lignins--natural antioxidants." Bioresour Technol **95**(3): 309-317.

Dizhbite, T., G. Telysheva, V. Jurkjane and U. Viesturs (2004). "Characterization of the radical scavenging activity of lignins—natural antioxidants." Bioresource Technology **95**(3): 309-317.

Ekielski, A., T. Żelaziński, P. K. Mishra and J. Skudlarski (2021). "Properties of biocomposites produced with thermoplastic starch and digestate: Physicochemical and mechanical characteristics." Materials **14**(20): 6092.

Figueiredo, P., K. Lintinen, J. T. Hirvonen, M. A. Kostiainen and H. A. Santos (2018). "Properties and chemical modifications of lignin: Towards lignin-based nanomaterials for biomedical applications." Progress in Materials Science **93**: 233-269.

Fukuzaki, S. (2006). "Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes." Biocontrol Sci **11**(4): 147-157.

Kumar, A., B. Biswas, K. Saini, A. Kumar, J. Kumar, B. B. Krishna and T. Bhaskar (2020). "Effect of hydrogen peroxide on the depolymerization of prot lignin." Industrial Crops and Products **150**: 112355.

Mayer, A. and G. Miskelly (2023). "Chemical decontamination of methamphetamine and ephedrine using household hypochlorite bleach." Forensic Chemistry **36**: 100538.

Qian, Y., X. Zhong, Y. Li and X. Qiu (2017). "Fabrication of uniform lignin colloidal spheres for developing natural broad-spectrum sunscreens with high sun protection factor." Industrial Crops and Products **101**: 54-60.

Seabra, J. E. A., L. Tao, H. L. Chum and I. C. Macedo (2010). "A techno-economic evaluation of the effects of centralized cellulosic ethanol and co-products refinery options with sugarcane mill clustering." Biomass and Bioenergy **34**(8): 1065-1078.

Ugartondo, V., M. Mitjans and M. P. Vinardell (2008). "Comparative antioxidant and cytotoxic effects of lignins from different sources." Bioresource Technology **99**(14): 6683-6687.

Wang, J., Y. Deng, Y. Qian, X. Qiu, Y. Ren and D. Yang (2016). "Reduction of lignin color via one-step UV irradiation." Green Chemistry **18**(3): 695-699.

Wen, J. L., S. L. Sun, B. L. Xue and R. C. Sun (2013). "Quantitative Structures and Thermal Properties of Birch Lignins after Ionic Liquid Pretreatment." J. Agric. Food Chem. **61**(3): 635.

Widsten, P., T. Tamminen and T. Liitiä (2020). "Natural Sunscreens Based on Nanoparticles of Modified Kraft Lignin (CatLignin)." ACS Omega **5**(22): 13438-13446.

Wu, C., Y. Yang, K. Sun, D. Luo, X. Liu, H. Xiao, H. Bian and H. Dai (2023). "Lignin decolorization in organic solvents and their application in natural sunscreen." International Journal of Biological Macromolecules **237**: 124081.