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Solving the lignin waste crisis: Utilization of lignin to produce value- added antioxidant agents

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Abstract: Lignin is a major component of biomass that is considered a huge energy and carbon reserve. Aside from being the second most abundant polymer in nature, it is a major by- product of pulping, paper and other forestry- based industries with about 70 million tons per year. Due to it being highly biologically recalcitrant, lignin is underutilized and considered a waste product that is solely incinerated for power generation. Establishment of a market for lignin- based, value- added chemicals was a necessity. Because of its renewable nature, availability, and richness in phenolic component, however, it is considered as an attractive alternative for antioxidant activity. In our research, all types of extracted lignin exhibited a value ranging from 2.21 to 11.47 on the AAI assay suggesting high antioxidant activity. Furthermore, special chemical structures such as hydrogen atoms, double bonds between side chain carbons, as well as the presence of methoxyl groups can increase antioxidant activity by 2- 3 folds via means of radical scavenging. Finally, lignin extracted at higher temperature (~95C and greater) exhibited higher ORAC values compared to lignin extracted at lower temperature; ORAC values of 1072.32 to 3119.68 versus 1741.72 to 1745.11, respectively. This paper is going to shed more light on the lignin antioxidant properties, where it stems from, what affects it, as well as future directions and possible interdisciplinary potential improvement.

Keywords: Lignin; Antioxidant activity; Characterization of lignin; Chemical analysis; radical scavenging activity.

حل أزمة نفايات اللجنين: استخدام اللجنين لإنتاج مضادات أكسدة ذات قيمة مضافة

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المستخلص: اللكنين يعتبر جزي أساسي من الكتلة الحيوية والتي تعد مخزن عظيم للطاقة الكربونية. علاوة على كونه ثاني أكثر البوليمرات انتشارا في الطبيعة، اللكنين يمثل ناتج ثانوي من صناعات عديدة مثل الورق وغيرها من الصناعات المبنية على حراجة الغابات. بسبب تركيبته المعقدة، قرابة ال70 مليون طن من اللجنين تعتبر مخلفات مما يحد من امكانية الاستفادة منها ويؤدي لحرقها لتوليد الطاقة. لكن بسبب طاقة اللكنين الكامنة، امكانية تكريره، وفرته، واحتواءه على الفينولات، يوجد توجه كبير للاستفادة منه في أكثر من مجال لصنع مختلف الكيماويات ذات القيمة المضافة خصوصا فيما يعرف بمضادات الاكسدة. في هذا البحث، أظهرت كل مستخلصات اللكنين قيمة تتراوح بين 2.21 و 11.47 على فحص مضادات الاكسدة (AAI) و هو ما يعني قدرة عالية. علاوة على ذلك، بعض الهياكل الكيميائية مثل ذرات الهيدروجين، الروابط الثنائية لين ذرات الكربون الجانبية، وتواجد الميثوكسيلات يمكنها زيادة النشاط المضاد للأكسدة عن طريق الكسح الجذري. أخيرا، اللكنين المستخلص في درجات حرارة عالية تظهر قدرات مضادة للأكسدة على مقياس قدرة امتصاص جذور الاوكسجين اعلى من نظيرتها المستخلصة في درجات حرارة أقل. هذه القراءات تتراوح بين 1072.32 و 3119.72 للأولى مقارنة ب 1741.72 و 1745.11 على التوالي.

في هذا المقال سيتم تناول الخصائص المقاومة للأكسدة للكنين، من اين تستمد، ما الذي يمكن أن يؤثر على هذه الخصائص، وكيف يبدو مستقبل صناعة اللكنين .

الكلمات المفتاحية: اللكنين، مضادات الأكسدة، توصيف اللجنين، التحليل الكيميائي، نشاط الكسح الجذري.

Introduction.

Polymers are a broad class of materials composed from repeating units of smaller molecules referred to as monomers. Because of their strength, durability, and structure, polymers are utilized across a wide range of industries including pharmaceuticals, coating industry, material manufacturing, as well as in paper and hygiene products (Hulme & Cooper, 2012). Although naturally existing polymers are present in some of these industries and products, they are not as commonly used as their fossil- based counterparts (Lochab et al., 2014). With the commercialization and scale up of industrial products, the need to find a renewable source to meet society's increasing basic needs is inevitable. Lignin is said to present a promising opportunity to fulfil their rule as a renewable feedstock for bioenergy, chemicals and materials production (Wang et al., 2019).

Lignin is an aromatic biopolymer that is typically found in plants, especially lignocellulosic plants and accounts for approximately 300 billion tons in the globe with a CAGR of 7% (Dessbesell et. Al, 2020). Plants produce lignin by fixing CO2 to reduce sugars, converting them to three- dimensional polymer of monolignols, p- coumaryl (4- hydroxy phenyl, H), coniferyl alcohol (guaiacyl, G) and sinapyl alcohol (syringyl, S) (Ralph et. Al, 2019). While cellulose and hemicellulose are the polysaccharides that form the cell wall, lignin acts as the cement that holds the lignocellulosic fibers together, providing rigidity to the material (Sena- Martins et. Al, 2008). Furthermore, it does not only provide protection against sunlight and frost, it also acts as a barrier against invading microorganisms as well as the penetration of enzymes that destroy cell wall sectioning, initiating plant degradation process (Garcia et. Al, 2009). Figure 1 shows the general chemical structure of lignin.

Kraft lignin

Kraft pulping was first introduced in Germany in 1879 (Hu et al., 2018). Kraft lignin results from kraft pulping, the most common chemical pulping process, and is largely available from paper mills (Faustino et. Al, 2010). The process is based on the delignification of pulping by- products by action of strong alkaline solution (high pH~14), such as sodium sulphide and sodium hydroxide, at a high temperature of about 160- 170 °C (Faustino et. Al, 2010). This enhances reactions promoting the cleavage of lignin macromolecules, solubilization of smaller fragments, and the liberation of cellulosic fibers, resulting in a final solution is called black liquor composed of cooking chemicals and wood substances (Ela et. al, 2020). As a portion of the wood is completely dissolved into it, this black liquor is incinerated with recovery of inorganic material, that is the cooking chemicals, and the production of steam (Wallberg et. Al, 2006). It is worth noting that kraft lignin is produced at a rate of 40 million tons per year worldwide (Bajwa et. al, 2019). Kraft lignin is said to be soluble in alkali, have molecular weight of 100 to 3000, and often contaminated with sulfite (Table 1- b) (Espinoza- Acosta et al., 2016).

Sulfite lignin (Lignosulfonate)

Acid sulfite process was the first to be introduced in Germany in 1840 (Hu et al., 2018). The sulfite process is one that uses acidic delignification with an objective of depolymerizing lignin by sulfonation and hydrolysis. It uses a cooking liquor at pH of about 5 and 130- 160 ºC. Under these conditions, lignin is dissolved by applying SO_3

$2-$ or HSO_3

–, producing the following reactions:

$R1\text{- }O\text{- }R_2 \longrightarrow H\,R_1 + R_2OH$

$R1\,HSO_3 \longrightarrow R1SO_3{-}H$ (soluble)

The sulfonate salts are known to be soluble in water, therefore, provided sufficient sulfonate groups are attached to it, lignin can be washed out resulting in lignosulfonate. It is worth noting that lignosulfonate is produced at a rate of 8 to 10 million tons per year worldwide (Hu et al., 2018). Lignosulfonates are soluble in water, with molecular weight of 20, 000 to 50, 000, and sulfur impurities (table 1- b) (Espinoza- Acosta et al., 2016).

Organosolv lignin

Organosolv lignin come from solvent pulping, which is also known as sulfur- free lignin. It is said to be a great alternative to kraft and sulfite pulping because it is more environmentally friendly and economically more efficient; and was only available in industrial quantities in the 1990's (Bajwa et. Al, 2019). The product recovered from the pulping process include both organosolv lignin as well as other chemicals. The precipitation of lignin from the spent solvent involves the adjustment of concentrations, pH, and temperature. Organosolv lignin exhibits many interesting properties; it usually is high in purity, low molecular weight, highly soluble in organic solvent, partially insoluble in water, and very hydrophobic (Zijlstra et. Al, 2020). In Canada, the majority of initiatives are still at a laboratory or pilot scale with production rate of about 100 tons per year (Dessbesell et. Al, 2020). Table 1 shows purity and potential applications of various technical lignins. Organosolv lignins are said to be soluble in organic solvents, have molecular weights of 500 to 4000, and often contaminated with carbohydrates and ash. (table 1- b) (Espinoza- Acosta et al., 2016).

Soda lignin

Just like organosolv lignin, it is considered to be a non- sulfur containing lignin. It is obtained by treating lignocellulosic materials such as sisal, bagasse, and wheat straw with highly alkaline solutions such as sodium hydroxide. According to Doherty et al (2011), it is obtained under the same conditions as kraft lignin except for the presence of hydrogen sulfite anions. In this process, it is the hydrolytic cleavage of native lignin network that drives the extraction. Lignin is recovered with several steps including acid

precipitation, heating, and filtration (Espinoza- Acosta et al., 2016). Although its properties resemble that of kraft lignin, soda lignin does not contain sulfur, contain low quantity of hemicellulose, and high quantity of silicate and nitrogen. This process is usually adapted in paper factories using agricultural residues as feedstock (Lora & Glasser, 2002).

Business case:

Numerous review papers have addressed lignin research; though, a limited focus has been given to lignin commercial initiatives and its market applications potential (Ragauskas et. Al, 2014). Forestry related processes such as paper manufacturing and biofuel production are said to be the largest producers of lignin (Matsushita et al, 2009). Resulting lignocellulosic material including wood, agricultural and forestry residues is processed in biorefineries, where it is fractioned into cellulose, hemicellulose and lignin (Garcia et. Al, 2018). Because lignin is biologically recalcitrant, and typically need extensive treatment to be converted to accessible sugars or building block chemicals, the vast majority of this lignin by- product is treated as waste, burnt for steam and power, or used as source of products like resins and surfactants (Hu et. Al, 2018).

Recently, Canada in particular emerged as one of the world leaders in the forest industry, which results in the continuous production of lignin as a byproduct. According to (Vanholme et al. 2008), forest-using industries generate 50 million tons of lignin annually. Furthermore, with high demand for forest industry products including lumber, pulp, and paper, lignin increases to be produced and accumulated with its use is almost completely limited to energy generation for biomass transformation (Smolarski, 2012). This underutilization of lignin certainly poses a gap between lignin producers and market off-takers, which definitely slows down adequate market formation and penetration. Novel lignin application, therefore, need to be proposed and tested to create market specifications that would allow for by-produced, accumulated lignin to be utilized.

This project aims to propose a way to utilize this abundant byproduct lignin as a product with economic and industrial potential. We are going to use literature to investigate the candidacy of lignin, and its derivatives, as an antioxidant agent. The final goals are to (1) creating new industrial and economic opportunities, while (2) making the most use of the forestry industry to meet the increasing market demand and strengthen Canada's forest products sector.

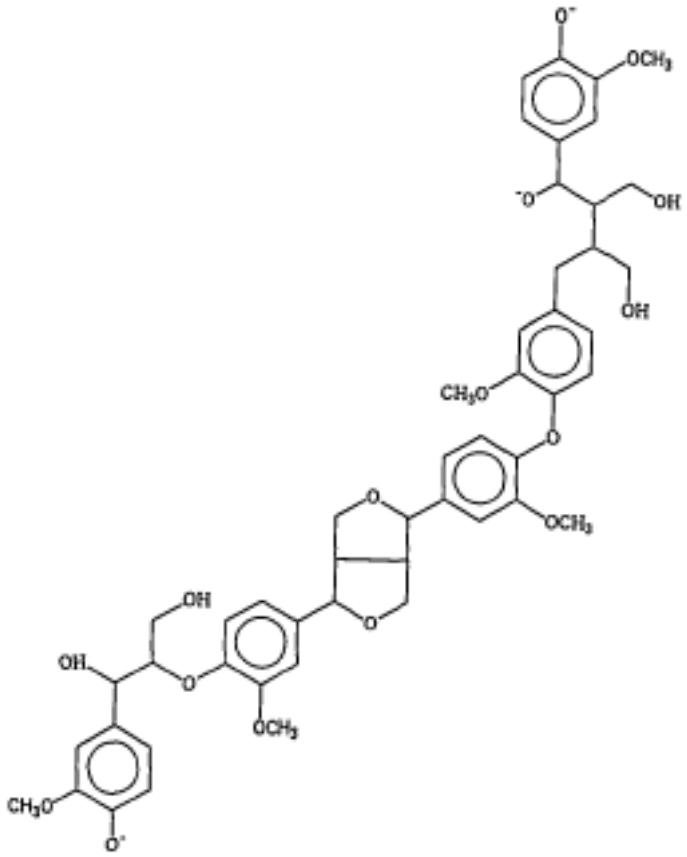


Figure (1) Chemical structure of lignin.

Table (1) a) Purity and potential applications of various technical lignins (Dessbesell et al. 2020); b) Physiochemical properties of different types of technical lignins (Espinoza- Acosta et al., 2016)

a)

Technical lignin	Purity	Potential products
Lignosulfonates	Medium	Additives in bitumen, vanillin, feedstock for refinery
Kraft lignin	Medium-high	Additives, biofuel, BTX, activated carbon, phenolic resins, carbon fibres, phenol
Organosolv	High	Activated carbon, phenolic resins, carbon fibres, vanillin, phenol derivatives
High-grade lignin (e.g. HL form the TMP technology)	Very high	Carbon fibres, vanillin, phenol derivatives

b)

	Kraft	Lignosulfonates	Soda	Organosolv
Solubility	Alkali	Water	Alkali	Organic solvents
Molecular weight (*Mw*)	100 to 3000	20,000 to 50,000	800 to 3,000	500 to 4000
Polydispersity	2.5 to 3.5	6 to 8	2.5 to 3.5	1.3 to 4.0
Impurities	Sulfur	Sulfur	Carbohydrates and ash	Carbohydrates and ash

Theory and methodology.

As lignin research is becoming a hot spot nowadays, its potential as a natural antioxidant agent is being investigated. For many reasons, especially the various possible obtaining processes, the structure of lignin still remains one of the most difficult biopolymers to characterize and, therefore, utilize. Recent advances in analytical chemistry and spectroscopy, however, greatly increased the body of knowledge of lignin as a crucial natural resource.

Chemical and Biological reagent (Kaur amd Uppal, 2015)

Lignin alkali with low sulfate content (commercial lignin), l- ascorbic acid (vitamin C), sodium hydroxide, 2, 2_- azobis (2- methylpropionamidine) dihydrochloride (AAPH), trifluoroacetic acid, 6-hydroxy- 2, 5, 7, 8- tetramethylchroman- 2- carboxylic

acid (Trolox), and fluorescein sodium were obtained fromSigma–Aldrich, Inc. (St. Louis, MO).

BactoTM tryptic soy broth (TSB) and BactoTM tryptic soy agar (TSA) were obtained from Dickinson and Company (Sparks, MD).

Ultrafiltration

A single- cjannel ceramic membrane ((TAMI Industries, model MSKTB 0251001, Nyons, France) with cutoff of 1000 Da, internal diameter of 0.006 m, and surface area of 0.0047 m^2 was used. The lignin solution was fed to the form a reservoir tank into the membrane using a gear pump (Micropump IDEX

Corporation, Vancouver, Canada), in which the crossflow velocity was measure using a flow meter (Floab Flödesprodukter AB, Model MS501, ML4- F1). the total amount withdrawn as permeate samples from the system was less than the total volume of the system by a factor of 0.001, the rest was circulated back to the system. The filtration occurred at TMP of 3 bar, temperature of 24 C, and crossflow velocity of 3.9 m/s. Initial feed was 6 kg of solution placed in a 10 liter feed tank, and it circulated for 12 hours with closed permeate valve closed (to reach steady- state). After collection of the permeates, they were stored in a cold room (ice bath ` 4 C). Lignin was then recovered by precipitation with dropwise addition of 6 M sulfuric acid while stirring to pH of 2.

Total Phenolic content (TPC)

The Folin- Ciocalteu method was used to determine total phenols content, where it was diluted with water (1:10, v/v) and then mixed with sodium carbonate aqueous solution. It is then mixed with aqueous solution of lignin and kept for 5 minutes at 50C. Following that, absorbance was measured at 760 nm. From the calibration curve of the gallic acid standard solution, total phenolic content of lignin solution can be determined.

Fourier- transform infrared spectroscopy (FTIR) Analysis:

It is a technique used to obtain infrared spectrum of absorption or emission while a spectrometer instantly and continuously collects data over a wide spectral range. FITR spectra of numerous lignin fractions were determined between 4000 and 400 /cm with resolution of 4 /cm. A total of 45 to 128 scans were recorded using Thermo Nicolet 670 spectrometer using single bounce ATR- FTIR spectroscopy. Lignin fractions were mixed with KBr and ground before analysis and peak areas were determined using different software including Omnic 7.0. the results are bands corresponding to the different functional groups. Spectrum was compared to those of standard compounds such as vitamin C and commercial lignin.

Nuclear magnetic resonance spectroscopy (NMR) Analysis

Also referred to as magnetic resonance spectroscopy and represents a technique by which local magnetic fields around atomic nuclei can be observed. NMR signal was produced by excitation of samples placed in a magnetic field by radio waves. This was then dedected with sensitive radio receivers. Due to intermolecular magnetic field around atoms of molecules, the resonance frequency changes, giving detailed insight to the electronic structures of both the molecule and its functional groups. Because magnetic fields are unique to different compounds, NMR is considered the definitive method to identify chemical compounds, where the resonant frequency, energy of radiation absorbed, and the intensity of the signal are said to proportional to the magnetic field strength.

High Pressure Liquid Chromatography- mass spectrometry (HPLC/MS)

It is an analytical technique that utilizes the physical separation abilities of HPLC and the mass analysis abilities of mass spectrometry (MS) leading to the enhancement of both techniques' capabilities. Liquid chromatography gives the ability to separate mixtures with multiple component, while mass spectrometry provides the ability to structurally identify individual components with high specificity and sensitivity. While the LC part is a pressurized liquid system, the MC analyzer operates under vacuum (10^- 6 torr / 10^- 7 Hg). They, therefore, need an interface to connect the two fundamentally incompatible systems.

High- performance liquid chromatograph (HPLC) (Waters 2695) equipped with a photodiode array detector (Waters 996) and an autosampler (Waters 717 Plus) and coupled to a quadrupole time- of- flight mass spectrometer (Q- TOF- MS) (Waters Corp., Milford, MA, USA) was used in the experiments. Mobile phase consisted of 1 mL L−1 acetic acid in high- purity water and 1 mL L−1 acetic acid in methanol with a 75 minute linear gradient.

Scanning electron microscopy (SEM):

Samples were coated with gold powder, after which SEM (MX2600FE) was used to determine the particle morphology of nanoscale lignin as well as non- nanoscale lignin. The observation was carried out under 1, 000x and 50, 000x.

Determination of antioxidant activities

Hydrophobic oxygen Radical Absorbance Capacity (ORAC)

Aliquot of diluted lignin fractions and other samples acting as calibration solutions used for comparison were added to 96- well bottom reading microplate. Fluorescent solution (150 um at 4.0x10^- 6 mM) was added to each well and incubated for 30 minutes at 37C. Following that, 25 ul of AAPH solution at 153 mM was added to each well as a source of peroxyl to start the reaction. Programmed to read fluorescence, the microplate reader recorded readings with excitation wavelength of 485 nm and emission wavelength of 520 nm every one minute for a period of one hour.

Radical Scavenging Activity: 1, 1- diphenyl- 2- picrylhydrazyl (DPPH*):

It uses 1, 1- diphenyl- 2- picrylhydrazyl (DPPH*) as a reactive free radical. Reaction between this radical and our fraction is a measure of the ladders scavenging ability. Although reactivity of DPPH* is much lower than that of oxygen- containing free radical agents, it is used as a standard method in antioxidant research. The free radical scavenging activity was expressed as the concentration required for 50% inhibition of free radicals (IC50). Hence, al lower IC50 value is associated with higher radical

scavenging activity of a sample. The process can be analyzed and followed spectrophotometrically as the purple color of DPPH* tend to fade in presence of antioxidant agents. Below is the suggested reaction:

Lignin + DPPH* ➔ [Lignin. DPPH*] ➔ Inactive products (Dizhbite (2004)

This method was developed by Brand- Williams in 1995, where lignin samples dissolved in dioxane/water (90:10, v/v) were mixed with 3.9mL of a 6°x10^−5 mol/L DPPH solution at 518 nm. The results were showed in TAC%, which is the percentage respect to the reduction in absorbance observed for DPPH* at t=0 minutes. The DPPH* radical scavenging activity was then calculated using the following formula:

DPPH* scavenging Activity (%) = [(A0 − A)/A0] x 100' where A0 is the absorbance of control and A is the absorbance of lignin sample.

ABTS+ assay

Assessed the capacity of lignin to to reduce the ABTS radical. Is a newer method published by (Baltrusaityte et al., 2007), where antioxidant activity is measured using lambda 650 UV/VIS spectrometer. I also use the idea of IC50. ABTS+ is usually diluted with phosphate buffer saline (PBS) or 50% (v/v) ethanol- water to get to an absorbance of about 0.800 at 734 nm. It is then mixed with respective fractions (usually 10 mg/ml of agent in 3 ml of ABTS+).

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was developed by Benzie and Strain in 1996 and it measures the reduction of ferric 2, 4, 6- tripyridyl- s- triazine (TPTZ). The results appear in a coloured product. The absorbance is usually measured at 610 nm with a spectrometer and then compared to a standard (commonly ascorbic acid). Therefore, the results are expressed as ascorbic acid equivalent (AAE).

Results and discussions.

Biobased phenolic compounds to use as building blocks for polymers synthesis and antiradical agents is increasingly in demand (Majira et al., 2019). The effective- ness of antioxidant or free radical scavenging capacity of phenolic compounds mainly depends on the lignocellulosic material and extraction procedure as well as presence and position of phenolic groups, especially hydroxyl groups, in the aromatic ring.

Crude extraction phenolic content

Phenols derived from biomass sources are said to be valuable chemicals (Kai et al., 2016). They can be used in the production of different kind of adhesives, synthesis of polymers, as well as some pharmaceuticals and nutraceuticals with different properties. For the most part, crudes are usually used rather than phenolic compounds as it is more economically logical.

In the present study done by Faustino et. Al (2010), ethyl acetate was used for the extraction of black and sulphite liquors. It was chosen for being a good solvent for the extraction of phenolic fractions, as well as its ability to be recovered by vacuum evaporation leaving only the obtained crude extracts (Radoykova et al., 2013). Extracts were then run onto HPLC/MS using 10% chloroform ethyl acetate as an eluent.

PH affects the amount of phenolics in the extracts

The Folin- Ciocalteu method was used to analyze fractions in terms of mass and total phenolic content (TPC) and spectrophotometric results are shown in figure 2. Looking at the figure, it can be seen that the highest phenolic content was obtained when kraft black liquor was extracted at pH of 6, giving a value of roughly 181.6 mgGAE/g. This was surprising because the extracted mass at the same pH (pH 6) was the second lowest, suggesting no linear relationship between mass and phenolic content. As for sulphite liquor, extract at pH of 6 showed the highest TPC value at 114.2 mgGAE/g.

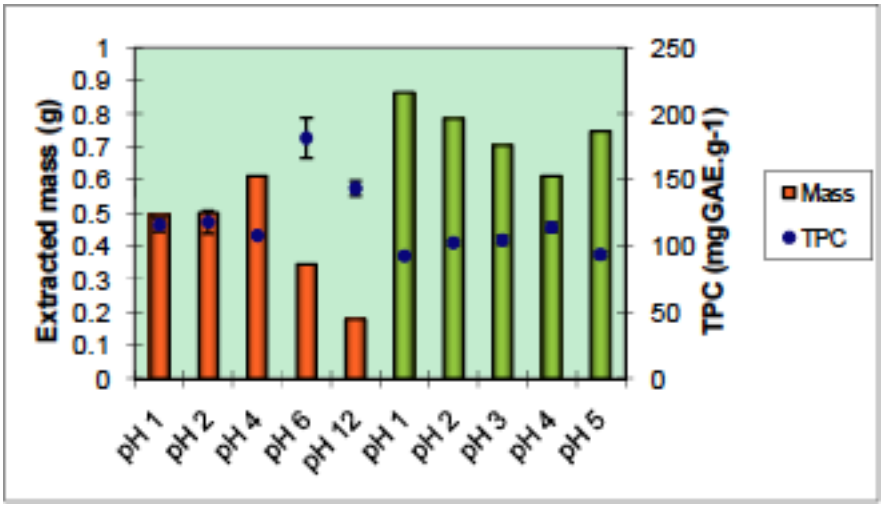


Figure (2) Total phenolic content of black liquor crude extracts at several pH: kraft (orange columns), and sulphite (green columns).

Radical Scavenging activity and antioxidant activity index (AAI)

Thanks to its hindered phenolic structure, lignin is said to have the ability to function as a free radical scavenging antioxidant activity (Kabir, 2017). Radical scavenging activity is often determined by the use of 1, 1- diphenyl- 2- picrylhydrazyl (DPPH*) as a radical. Table 2 shows the AAI values of kraft and sulphite liquor extracts under different DPPH* concentrations, namely 31.6, 49.0, and 78.8 ug/mL, as well as different pH values, 1 through 12. In the case of kraft extracts, and despite the low mass, the highest mean AAI value is obtained under pH of 6 (~3.41) and it increases with the increase in DPPH* concentrations. In the sulphite extract series, however, the highest AAI value was obtained at a pH of 2 (~3.29). Surprisingly, based on the scale proposed by Scherer and Godoy, there is no significant difference between all AAI values obtained at different pH values. According to Scherer and Godoy (2009),

antioxidant activity is said to be poor when AAI is lower than 0.5 (AAI<0.5), moderate when AAI is between 0.5 and 1.0 (0.5<AAI<1.0), and very strong when AAI is greater than 2.0 (AAI>2.0). Therefore, all obtained extracts had very strong antioxidant activity.

Table (2) Values of antioxidant activity index (AAI) with different final concentrations of DPPH* for crude extracts from kraft and sulphite black liquors at different pH values. (Faustino et al., 2010)

Kraft black liquor					Sulphite black liquor				
pH	DPPH conc. ($\mu g.mL^{-1}$)	Mean IC_{50}	Mean AAI [a]	Total Mean AAI [a]	pH	DPPH conc. ($\mu g.mL^{-1}$)	Mean IC_{50}	Mean AAI [a]	Total Mean AAI [a]
	31.6	21.51	2.17 ± 0.03			31.6	12.82	2.54 ± 0.02	
1	49.0	29.15	2.16 ± 0.25	2.20 ± 0.18	1	49.0	18.31	2.88 ± 0.02	2. 92 ± 0.34
	78.8	34.03	2.27 ± 0.23			78.8	26.99	3.32 ± 0.06	
	31.6	22.03	2.10 ± 0.12			31.6	11.36	2.91 ± 0.03	
2	49.0	24.02	2.57 ± 0.02	2.48 ± 0.30	2	49.0	16.35	3.26 ± 0.01	3.29 ± 0.35
	78.8	30.92	2.76 ± 0.02			78.8	24.38	3.71 ± 0.02	
	31.6	19.64	2.02 ± 0.08			31.6	12.36	2.67 ± 0.03	
4	49.0	22.84	2.35 ± 0.01	2.31 ± 0.25	3	49.0	18.67	2.89 ± 0.04	2.98 ± 0.31
	78.8	30.04	2.58 ± 0.07			78.8	27.41	3.37 ± 0.06	
	31.6	13.19	3.00 ± 0.01			31.6	9.33	2.88 ± 0.06	
6	49.0	15.16	3.47 ± 0.02	3.41 ± 0.33	4	49.0	15.65	3.01 ± 0.02	3.12 ± 0.28
	78.8	20.80	3.76 ± 0.04			78.8	21.95	3.48 ± 0.01	
	31.6	10.44	2.61 ± 0.03			31.6	10.88	2.50 ± 0.06	
12	49.0	18.67	2.62 ± 0.03	2.74 ± 0.20	5	49.0	20.59	2.34 ± 0.36	2.60 ± 0.34
	78.8	26.07	3.01 ± 0.05			78.8	25.99	2.96 ± 0.11	

Source of fractions

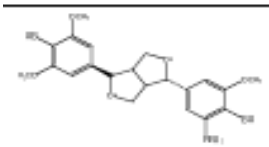
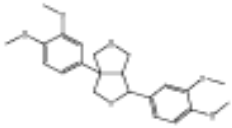
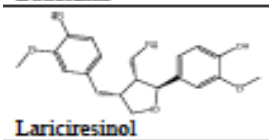
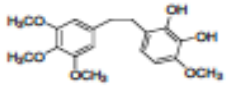
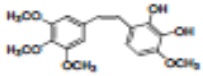
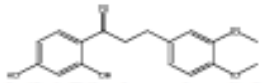
Ten fractions, K1 through K6 and S1 through S4, prepared from two sources, kraft and sulphite (Faustino et al., 2010). Fractions K1, K2, K3 and K4 were insoluble in methanol, K5 and K6 had normal methanol solubility behaviour. These fractions were obtained under their ideal pH, 6 for kraft (K) and 5 for sulphite (S). Similar to the crudes, table 2 the mass and TPC of the separated fractions. It can be seen that overall TPC values were much greater in sulphite fractions than in kraft fractions.

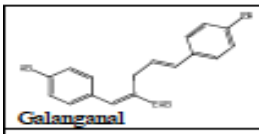
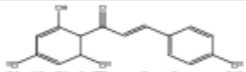
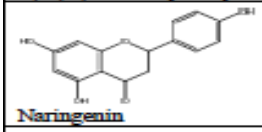
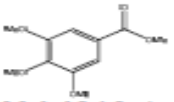
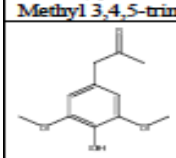
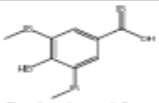
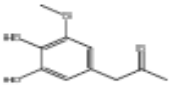
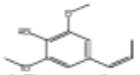
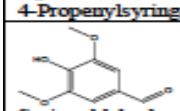
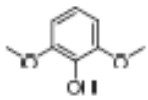
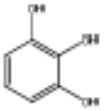
Furthermore, looking at table 2, it can be seen that fractions separated from sulphite liquor exhibited an AAI value ranging from 2.85 to 11.47. On the other hand, fractions separated from kraft liquor exhibited an AAI value ranging from 2.21 to 9.14. Overall, according to our proposed scale, all fractions showed very high antioxidant activity (AAI>2.0). It is perhaps worth mentioning that the overall better performance in the case of sulphite liquor is suspected to be due to variation between the two pulping processes, which leads to less degraded phenol structures (Aadil et al., 2014). Table 4 shows compounds identified in respective fractions along with their structure, formula, and molecular weight.

Table (3) characteristics of separated fractions from kraft and sulphite black liquors. (Faustino et al., 2010)

Kraft black liquor pH = 6				Sulphite black liquor pH = 5			
Fraction	Rf [a]	Mass of the fraction (g)	TPC [b] (mgGAE.g-1)	Fraction	Rf [a]	Mass of the fraction (g)	TPC [b] (mgGAE.g-1)
K2	0.61	0.0492	293.5 ± 8.71	S1	0.76	0.4897	363.1 ± 11.13
K3	0.38	0.1852	146.9 ± 15.94	S2	0.51	0.1654	612.4 ± 22.64
K5	0.20	0.2487	198.1 ± 2.08	S3	0.32	0.2363	1099.6 ± 2.48
K6	0.05	0.1529	91.6 ± 0.17	S4	0.19	0.9812	966.8 ± 7.92

Table (4) Compounds identified by HPLC/MS compared with literature (Faustino et al., 2010).

Possible structure/name	Formula	Molecular weight	Exact mass found	Reference	Fractions
 Epi-syringaresinol	$C_{22}H_{26}O_8$	418.44	418.16	[24]	K5; K6; S4
 Eudesmin	$C_{22}H_{26}O_6$	386.44	386.17	[25]	S3
 Lariciresinol	$C_{20}H_{24}O_6$	360.40	360.16	[26]	K6
 3-Methoxy-6-(3,4,5-trimethoxyphenethyl)benzene-1,2-diol	$C_{18}H_{22}O_6$	334.36	334.14	[27]	K3
 (*Z*)-3-Methoxy-6-(3,4,5-trimethoxystyryl)benzene-1,2-diol	$C_{18}H_{20}O_6$	332.35	332.13	[27,28]	K3; K5; S3
 1-(2,4-Dihydroxyphenyl)-3-(3,4-dimethoxyphenyl)-propan-1-one	$C_{17}H_{18}O_5$	302.32	302.12	[29,30]	K3; S3

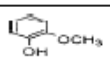
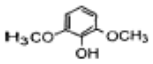
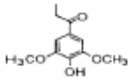
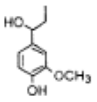
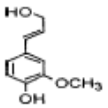
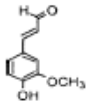
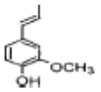
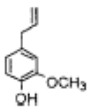
 Galanganal	$C_{18}H_{16}O_3$	280.32	280.10	[31]	K6; S5
 2',4',6',4-Tetrahydroxydihydrochalcone	$C_{15}H_{14}O_5$	274.27	274.09	[32]	S4
 Naringenin	$C_{15}H_{12}O_5$	272.25	272.07	[33]	K3
 Sulphur	S_8	256.52	255.78		K1
 Methyl 3,4,5-trimethoxybenzoate	$C_{11}H_{14}O_5$	226.23	226.09	[34]	S4
 Syringylacetone	$C_{11}H_{14}O_4$	210.23	210.09	[35]	K3; S1; S2; S3; S4
 Syringic acid	$C_9H_{10}O_5$	198.17	198.05	[36]	S4
 Acetosyringone	$C_{10}H_{12}O_4$	196.20	196.07	[35]	K3; K4; K5; S2
 4-Propenylsyringol	$C_{11}H_{14}O_3$	194.23	194.09	[35]	K5
 Syringaldehyde	$C_9H_{10}O_4$	182.17	182.07	[35,37]	K3; K4; K6; S1; S2; S4
 Syringol	$C_8H_{10}O_3$	154.16	154.06	[35,38]	K2
 Benzene-1,2,3-triol	$C_6H_6O_3$	126.11	126.03	[37]	K5

Functional groups and Radical Scavenging activity

The electronic structure of phenolic compounds, such as flavonoids and catechins, play an important role in capturing oxygen containing free radicals including OH*, RO*, ROO*, and O2*. This leads to the reduction of the oxidative stress. Therefore, the effectiveness of antioxidant activity of a compound is a measure of its free radical scavenging capacity (Kabir, 2017).

To assess the free radical scavenging activity of a compound, 1, 1- diphenyl- 2- picrylhydrazyl (DPPH*) is used. Using commercially available model lignin compounds; 2- methoxyphenol (guaiacol), 2, 6- dimethoxyphenol (syringol), 2- methoxy- 4- (prop- 2- enyl)phenol (eugenol), 4- allyl- 2- methoxyphenol, (2E)- 3- (40- hydroxy- 30- methoxyphenyl)prop- 2- enal (coniferyl aldehyde), 4- ((1E)- 30- hydroxyprop- 10- enyl)- 2- methoxyphenol (coniferyl alcohol). The following compounds: 2- methoxy- 4- propylphenol (propyl quaiacol), 1- (40 hydroxy- 30- methoxyphenyl)propan- 1- one (guaiacyl propanone- 1), 4- (10- hydroxypropyl)- 2- methoxyphenol (guaiacyl propanol- 1) and 4((1E)prop- 10- enyl)- 2- methoxyphenol (isoeugenol), antiradical efficacy was assessed. Compounds with their respective structure, antiradical power (ARP), as well as number of DPPH* moles reduced by one mole of compound are summarized in table 2. It is evident that the amount of non- etherified OH phenolic groups increases the overall scavenging activity. However, looking at the table, it can be seen that for some compounds, namely II, VI, VII and X, the number of reduced DPPH* is significantly higher that the number of their phenolic hydroxyl groups. This suggests that there are other factors in play. According to Dizhbite et. Al (2004), hydrogen atoms, being benzylic or directly bound to aromatic rings, can also react with DPPH* radicals contributing to the compound antiradical efficacy. Furthermore, the addition of methoxyl group (CH3O) in the ortho position seems to have a positive effect on the DPPH* scavenging ability. According to Dishbite et al. (2020), it contributes to the stability of the phenoxyl radical by means of resonance and hindering their propagation, thereby increasing antiradical activity. This is shown by the increase in ARP from 2.6 in I to 3.6 in II and from 0.2 in III to 0.5 in IV. Similarly, double bonds between the outmost carbons in the side chain seem to increase the antiradical activity even further (Table5, X and IX).

Table (5) Characterization of radical scavenging efficacy of lignin related compounds (Dizhbite et al., 2004).

No.	Compound		ARP[a]	Number[b] of reduced DPPH·
I	2-Methoxyphenol (guaiacol)		2.6	1.3
II	2,6-Dimethoxyphenol (syringol)		3.6	1.8
III	1-(4′hydroxy-3′-methoxyphenyl)propan-1-one (guaiacyl propanone-1)		0.2	<0.1
IV	1-(4′hydroxy-3′,5′-dimethoxyphenyl)propan-1-one (propiosyringone)		0.5	0.2
V	2-methoxy-4-propylphenol (propyl quaiacol)		3.5	1.75
VI	4-(1′-hydroxypropyl)-2-methoxyphenol (guaiacyl propanol-1)		3.0	1.5
VII	4-((1E)-3′-hydroxyprop-1′-enyl)-2-methoxyphenol (coniferyl alcohol)		4.2	2.1
VIII	(2E)-3-(4′-hydroxy-3′-methoxyphenyl)prop-2-enal (coniferyl aldehyde)		1.9	0.97
IX	4((1E)prop-1′-enyl)-2-methoxyphenol (isoeugenol)		2.2	1
X	2-methoxy-4-(prop-2-enyl)phenol (eugenol)		4	2

Preparation condition

Extraction method

Lignin samples with higher purity, total phenolic content, and lower molecular weight are said to have higher antioxidant activity (An et al., 2017). These two characteristics, however, are strongly dependent on the obtaining method (Ugartondo et al., 2008). According to Naseem et. Al (2016), these has been extensive research on the effect of the use of water, organic solvents, and alkaline for extraction on the total phenolic content, molecular weight and purity, and therefore, the free radical scavenging and antioxidant activity.

In an experiment performed by Aadil et. Al (2014), nine different fractions of lignin extracted by alkali, hot water, and organosolv methods from Acacia wood powder; LS- lignosulphonic acid, A1_alkali

lignin 0.1 N NaOH, A2- alkali lignin 0.2 N NaOH, A3- alkali lignin 0.3 N NaOH, A4- alkali lignin 0.4 N NaOH, HWL- hot water extracted lignin, AEL- acetone extracted lignin, EEL- ethanol extracted lignin, MEL- methanol extractedlignin, and PEL- propanol extracted lignin, were studied for antioxidant activity. These fractions were assessed using total phenol content (TPC), antioxidant activity (DPPH*), ABST, FRAP, hydrogen oeroxide scavenging (HPS), and reducing power (Table 6) (Aadil et. Al, 2014). Looking at the table, it can be noticed that phenolic content for both organosolv and hot water extracted fractions were higher than for alkali extracted lignin fractions. Furthermore, the lowest DPPH, associated with lignosulfonic acid fractions, is suspected to be due to its high molecular weight, which enhances heterogenicity. On the other hand, besides the large number of active functional groups that can act as electron donors, the high radical scavenging activity associated with organosolv lignin are due to its low molecular weight. This is supported by FTIR analysis (Fig. 3). The high radical scavenging activity of ethanol (EEL) and acetone (AEL) fractions are proposed to be due to the presence of C=O groups appearing around 1700/cm on FTIR spectrum that can donate electrons to radicals and provide stability to different compounds. The effect of the presence of active functional groups is additively combined with low molecular weights. Both of these characteristics, presence of functional groups and molecular weight, are largely dependent on extraction and fractioning processes. It was found that alkaline processes resulted in lignin with hemicellulose contamination compared to organosolv (Garcia et al., 2010) & (Dizhbite et al., 2004). According to Garcia et al (2012), this results in the generation of numerous hydrogen bonds between carbohydrates and phenolic groups, and in turn, lower antioxidant activity. It is, however, worth noting that evidence suggest that the improvement of the antioxidant power of the alkaline lignin samples are possible via use of secondary intensification process such as ultrafiltration, differential precipitation, and purification techniques. These techniques can reduce heterogenicity by removing hemicellulosic impurities and lower molecular weight (Aadil et al., 2014).

The overall results were in agreement with Garcia et al (2012). Out of the different fractionations processes investigated including organosolv, autohydrolysis, and alkaline samples, it was established that organosolv fractions showed the highest radical scavenging activity (Garcia et al., 2012). Espinoza- Acosta et al (2016), concluded that lignin samples with more phenolic hydroxyl groups, high homogeneity, lower molecular weight, less aliphatic hydroxyl groups, and narrow polydispersity showed higher antioxidant activity. It was proposed that low molecular weight lignin fractions tend to have higher amounts of aromatic hydroxyl as well as higher homogeneity than higher molecular weight fractions. And, therefore, higher antioxidant activity (Espinoza- Acosta et al., 2016).

Since purity and low molecular weight have been regarded as a prerequisite for lignin antioxidant activity (An et al., 2017), Aminzadeh and colleagues (2018) proposed the use of ceramic membrane with a molecular weight exclusion of 100 kDa. The resulting fractions consisted of small lignin dimers and oligomers with high content of methoxy- groups, which is suggested to enhance the antioxidant activity.

The antioxidant capacity of the resulting low molecular weight fractions was evaluated using three different assays; ABTS+, ORAC, and DPPH* (Table). The low molecular weight lignin fraction demonstrated significantly higher antioxidant activity on both ABTS+ (lower IC50 values) and ORAC assays, but lower on DPPH*. This discrepancy can be primarily due to the difference in the mechanisms of radical scavenging reactions in the tests (Ponomarenko et. Al, 2014). DPPH* relies on the hemolytic O- H bond cleavage mechanism, described in (Mishra et. Al, 2012). ABTS+ and ORAC on the other hand, rely on a SPLET mechanism based on the heterolytic dissociation of phenolic OH groups, described in (Zulueta et. Al, 2009). Furthermore, although lower overall, 2D NMR analysis showed that low- molecular- weight lignin fractions demonstrated a much higher proportion of non- condensed phenolic OH groups compared to regular sample (Table 8).

Table (6) Phenol contents, antioxidant activity on DPPH* radicals, ABTS radicals, FRAP value, hydrogen peroxide scavenging and reducing power of lignin fractions.

Lignin Fractions	TPC[1]	DPPH[2]	ABTS[3]	FRAP[4]	PMA[5]	HPS[6]	RP[7]
LS	100.27 ± 5.6^{f}	149.96 ± 4.4^{d}	2.70 ± 0.1^{a}	43.05 ± 8.5^{c}	67.96 ± 2.2^{f}	178.61 ± 1.2^{i}	19.8 ± 1.3^{f}
A1	232.48 ± 6.2^{d}	83.85 ± 0.4^{a}	2.84 ± 0.7^{a}	165.36 ± 19.7^{b}	120.55 ± 6.7^{d}	139.2 ± 0.7^{f}	99.4 ± 1.2^{d}
A2	73.01 ± 3.2^{g}	103.86 ± 4.4^{b}	3.25 ± 0.09^{c}	26.01 ± 4.8^{c}	108.88 ± 6.7^{d}	107.67 ± 1.4^{b}	$20.7 \pm .0.4^{f}$
A3	102.27 ± 3.5^{f}	110.48 ± 1.2^{b}	3.15 ± 0.05^{b}	55.92 ± 7.1^{c}	97.77 ± 6.4^{e}	175.08 ± 0.8^{h}	35.6 ± 1.7^{e}
A4	87.74 ± 1.9^{g}	128.21 ± 8.5^{c}	3.95 ± 0.13^{d}	40.64 ± 8.5^{c}	71.10 ± 2.2^{f}	162.20 ± 0.5^{g}	25.3 ± 0.6^{f}
HWL	295.21 ± 7.3^{c}	81.46 ± 0.9^{a}	2.78 ± 0.11^{a}	241.94 ± 19.0	175.92 ± 6.1^{c}	161.23 ± 0.9^{g}	125.8 ± 2.2^{c}
AEL	343.28 ± 3.2^{b}	79.89 ± 0.07^{a}	2.95 ± 0.14^{a}	294.16 ± 17.9^{a}	342.2 ± 12.9^{a}	114.7 ± 1.6^{d}	196 ± 0.9^{a}
EEL	229.54 ± 9.07^{d}	81.72 ± 00^{a}	2.77 ± 0.15^{a}	157.40 ± 55.5^{b}	167.58 ± 2.5^{c}	121.4 ± 0.8^{e}	138.5 ± 7.4^{b}
MEL	393.30 ± 9.2^{a}	84.58 ± 3.5^{a}	nd	273.23 ± 12.2^{a}	211.47 ± 1.9^{b}	110.7 ± 1.2^{bc}	140.6 ± 1.7^{b}
PEL	208.28 ± 4.1^{e}	82.64 ± 0.6^{a}	nd	66.47 ± 10.5^{c}	54.44 ± 3.8^{f}	101.8 ± 0.7^{a}	2.33 ± 0.3^{g}

Values represent mean ± standard deviation (SD) of three independent experiments (n = 3). Values within in a column followed by different lower case letters are significantly different according to ANOVA (Tukey test). nd—not determined.

[1]Total phenol content expressed as concentration of polyphenol (μg) in term of gallic acid equivalent (GAE) per mg of extracted lignin fractions.

[2]Concentration of test compound required to scavenge 50% inhibition of DPPH radical.

[3]Concentration of test compound required to scavenge 50% inhibition of ABTS radical.

[4]FRAP value was expressed as equivalent of gallic acid (μmol).

[5]Phosphomolybdate assay of each lignin fractions were expressed as equivalents of ascorbic acid (mmol/100 mg of sample).

[6]Concentration of test compounds required to scavenge 50% of hydrogen peroxide (HPS-CUPRAC assay).

[7]Reducing power of all extracted lignin fractions.

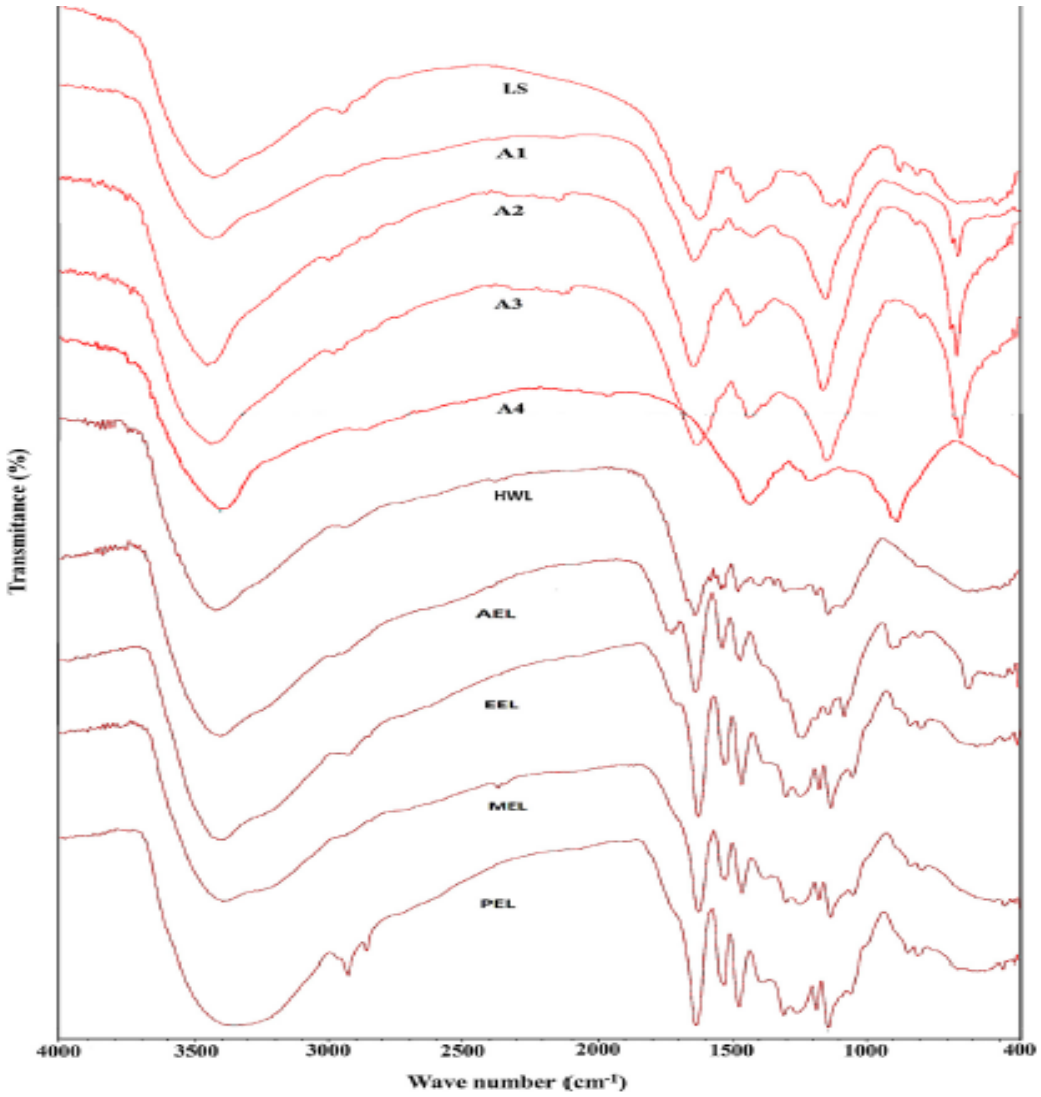


Figure (3) FTIR spectra of nine different lignin fractions. LS- lignosulphonic acid, A1_alkali lignin 0.1 N NaOH, A2- alkali lignin 0.2 N NaOH, A3- alkali lignin 0.3 N NaOH, A4- alkali lignin 0.4 N NaOH, HWL- hot water extracted lignin, AEL- acetone extracted lignin, EEL- ethanol extracted lignin, MEL- methanol extractedlignin, and PEL- propanol extracted lignin.

Table (7) Antioxidant properties of non- fractionated lignin and its low molecular weight fraction

Radicals	Lignin samples	
	LignoBoost lignin	Low-Mw fraction
DPPH·, IC_{50} (mg L^{-1})	23.7 ± 0.9	32.8 ± 1.0
$ABTS^{\cdot+}$, IC_{50} (mg L^{-1})	5.5 ± 0.1	3.9 ± 0.2
ORAC assay (TE mmol g^{-1}) [a]	9.09 ± 0.1	14.9 ± 0.2

Table (8) Content of functional groups determined using NMR (Aminzadeh et al., 2018)

Sample	Phenolic OH (mmol/g)		
	condensed	Non-condensed	Total phenolic
Lignoboost lignin	2 ± 0.04	2.27 ± 0.0	4.27 ± 0.04
Low-MW fraction	0.95 ± 0.05	2.85 ± 0.21	3.8 ± 0.27

Temperature and residue- solvent ratio

Just like extraction solvent, other extraction conditions such as temperature and residue- solvent ratio have an impact on the antioxidant activity (Dong et. Al, 2011). In their study, four extracts from residue of corn stover to ethanol under two different temperatures, 95c and 22c, as well as different residue- solvent ratio, ¼ and ½, were investigated (Table 9). The hydrophilic oxygen radical absorbance capacity (ORAC) assay was used to determine antioxidant activity. The results showed that, although lowest in yield, extract 1 (95c and ¼ ratio) exhibited a significantly higher ORAC value. This is in- line with literature (Vasquez- Olivo et. Al, 2019). according to Pan et al. (2006), at non- extreme conditions, lignin extracts prepared at elevated temperature, longer reaction times, and high dilutions generally exhibit higher antioxidant activity.

An increase in temperature is accompanied with enhanced delignification, that is the dissolution of lignin, thus a higher yield due to the enhanced cleavage of alpha and beta ether linkages between lignin structural units (Gilarranz et. Al, 2000). This is true up to a certain temperature (~195c) because excessive depolymerization reduces the recovery of lignin. An explanation is that excessive heating, especially with longer reaction time, is associated with formation of small fragments of lignin that are highly soluble in solvent and difficult to be recovered by precipitation processes resulting in low lignin yield (Pan et. Al, 2006).

Similarly, lower solvent concentration is associated with higher hydrogen ion concentration and, therefore, lower pH value. These conditions promote acid- mediated cleavage of alpha and beta linkages within the lignin (McDonough, 1993). Whereas a minimum solvent concentration is required, the maximum lignin concentration is generally observed at about 65% solvent. The increase of solvent concentration above 75% is associated with reduced lignin yield (McDonough, 1993). According to Pan et. Al (2006), this is due to the reduction of hydrogen ion concentration and higher pH; therefore, depressed delignification.

Table (9) Extraction conditions, yields, antioxidant capacities of lignin extracts, 1, 2, 3, 4, commercial lignin and vitamin C (Dong et al., 2011)

Sample	T (°C)	Time (min)	Ratio (w/v)	Yield (%)	ORAC value (μmol TE/g sample)	Total soluble phenolic (mg/g)
Extract 1	95	120	1/4	7.70 ± 0.41[a]	3119.68 ± 928.93[b]	200.40 ± 42.99[a]
Extract 2	95	120	1/2	14.46 ± 0.14[a]	1972.32 ± 784.21[c]	190.96 ± 3.18[a]
Extract 3	22	20	1/4	11.00 ± 0.15[a]	1745.11 ± 458.00[c]	179.63 ± 2.67[a]
Extract 4	22	20	1/2	13.82 ± 5.10[a]	1741.72 ± 930.34[c]	175.76 ± 37.91[a]
Commercial lignin					3516.72 ± 237.11[a]	165.50 ± 13.40[a]
Vitamin C					530.64 ± 22.37[d]	

Conclusions

There is currently a high amount of lignin being produced as by- products from different pulping refineries and it is estimated to be about 70 million tons each year. For lack of economic opportunity and absence of market, lignin is said to be underutilized, used only in in production of steam and power. Therefore, an establishment of a market in which lignin value- added products was essential for the sustainability of pulping and forestry- based industries to meet increasing demand. For its wide abundance and unique chemical structure, lignin presented itself as a molecule with incredible potential as an antioxidant agent.

There are typically three types of lignins based on the process by which they are obtained, kraft lignin, sulfite lignin and organosolv lignin. Total phenolic content is a key measure by which lignins can be characterized; interestingly, it was highly dependant on the pH under which the extraction occurs. The antioxidant activity is said to be carried out by means of free radical scavenging mechanism, by which specific phenolic groups react with and capture free radicals such as reactive oxygen species. Antioxidant Activity Index (AAI) assay showed that lignin extracts from different process showed varying AAI scores but no significant differences were observed. They are all said to have very high antioxidant activities.

Although phenolic OH content is a key factor in the radical scavenging mechanism, it is not the only factor at play. This was inferred from the fact that number of DPPH*s reduced by some fractions were significantly higher than number of phenolic OH groups in these fractions. It was shown that hydrogen atoms, as well as outmost double carbon bonds inside chain, can also contribute to antiradical activity by reacting with radicals. Furthermore, the addition of other groups can have an effect on the antioxidant activity. For example, the addition of methoxyl group (CH3O) seems to contribute to the stability of phenolic radicals by means of resonance and propagation hinderance, which enhances the antioxidant activity.

Besides phenolic content, chemical structure, and additions, purity and molecular weight tend to have an effect on antioxidant activity. These factors are dependant on the extraction process. Lignosufonate fractions tend to score low on the DPPH assay due to its high molecular weight, which contributes to its heterogeneity. Organosolv fraction, on the other hand, exhibited significantly high activity due to its low molecular weight, which also contribute to its homogeneity. Therefore, the effect of low molecular weight and active functional groups can be additive. Alkaline processes resulted in fractions

with hemicellulose contamination, and therefore, showed lower performance on the assay. This is crucial because numerous hydrogen bonds can occur between carbohydrates and phenolic groups leading to decrease scavenging activity.

Other extraction conditions such as temperature and residue- solvent ratio can also have an impact on antiradical activity. It was shown that lignin extracts prepared at elevated temperature exhibit higher antioxidant activity because it is associated with higher delignification rate, and therefore higher yield. This however is only true up to a certain temperature where delignification behaviour would change. It was also shown that lignin fractions prepared in high dilutions, that is lower solvent concentration, generally exhibit higher antioxidant activity because it is associated with lower pH value, and therefore, enhanced delignification. This only holds above a certain concentration minimum, under which this is untrue.

Recommendations.

As lignin proved its effectiveness as an antioxidant agent with excellent potentials, investigation of ways to improve the lignin antioxidant capacity seems to be the only logical step. Majira et al (2019) tackled the enhancement of antioxidant activity of technical lignins by simply combining both the standard solvent fractioning techniques, using ethyl acetate (EA), butanone, and methanol extractions and ionic liquid treatment. The result was the stabilization of 28% of the ethyl acetate- insoluble fraction and the production of additional free phenols in all fractions (EA, butanone, and methanol) (Majira et al., 2019). This represented a step in the direction of sustainably producing a phenol- oligomers with antioxidant activities higher than those of commercial antioxidants (Majira et al., 2019). However, a detailed analysis of cost should be performed to assess the overall economic benefits of using this hybrid approach to produce such chemicals, taking into account additional cost, availability of technology, and antioxidant activity gains.

In addition, certain limitations can arise that makes the utilization of lignin rather complicated (Naseem et al., 2016). Properties of lignin can change in response to change in relative moisture level. According to Naseem et al (2016), This can lead to its free radical component to react with atmospheric oxygen. Furthermore, at certain moisture levels, lignin can have a transition temperature of 20 – 25 C, in which it acts a fragile glass. What makes this significant is that the common application temperature of lignin rages between 20 and 25 degrees, limiting the ability to utilize lignin. Unfortunately, existing technologies to overcome these limitations are either financially costy or thermodynamically illogical (Naseem et al., 2016).

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