Antioxidant, Antimicrobial, and Antimutagenic Properties of Technical Lignins and Their Applications

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At present, more than 70 million tons per year of technical lignins are obtained from cellulose pulping and lignocellulosic refineries (*e.g.,* kraft, lignosulfonates, soda, and organosolv lignin). These lignins are commonly incinerated to produce steam and energy, and only a small part is used as an additive in various low volume and niche applications, such as dispersant, in concrete admixtures, as an adhesive and as a binder. Furthermore, the potential of technical lignins is considered to be beyond that of an inexpensive fuel or raw material to produce low added value products. The technical lignins consist of complex polyphenolic polymers that contain numerous chemical functional groups, such as phenolic hydroxyl, carboxylic, carbonyl, and methoxyl groups. The phenolic hydroxyl and methoxyl groups present in lignin reportedly possess various biological activities. The amount of data describing the biological activities of technical lignins has increased in the last 10 years. This review presents the most relevant research concerning the various biological activities (antioxidant, antimicrobial, antimutagenic, and others) of technical lignins. Additionally, the most promising and relevant applications are highlighted.

Keywords: Technical lignins; Biological activity; Antioxidant; Antimicrobial; Antimutagenic

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INTRODUCTION

Technical lignins are a group of complex phenolic polymers available in large quantities as by-products of the manufacture of cellulose pulp for paper and the production of ethanol from lignocellulosic biomass (Lora 2008). Despite the large amount of lignin produced (over 70 million tons per year), lignins are traditionally considered as bio-based waste material (Mansouri and Salvadó 2006). A small amount (approximately 2%) of technical lignins are used to produce low added value products (Gargulak and Lebo 2000; Lora and Glasser 2002), and the remaining are commonly burned as part of the treatment of the spent pulping liquors to obtain energy (Lora 2008). Because of their high availability and renewability, lignins represent an excellent source for the production of valuable functional molecules (Laurichesse and Averous 2014). Technical lignins contain numerous chemical functional groups, such as phenolic hydroxyl, carboxylic, carbonyl, and methoxyl groups (Adler 1977). The phenolic hydroxyl and methoxyl groups contained in lignin have been reported to be biologically active. One of the most studied properties of lignins is their antioxidant activity, (*e.g.*, their ability to act as radical scavengers), with the aim of using them as natural additives to replace synthetic or semi-synthetic compounds in cosmetics, pharmaceuticals, and polymeric formulations (Ugartondo *et al.* 2008; Vinardell *et al.* 2008; Lu *et al.* 2012b).

Different types of lignins possess antimicrobial, antioxidant (Dong *et al.* 2011), and UV absorption properties (Liu *et al.* 2014; Yu *et al.* 2015). Various investigations have suggested that lignins can be applied to stabilize food and feedstuff because of their antioxidant, antifungal, and antiparasitic properties (Baurhoo *et al.* 2008). Additionally, other properties such as anticarcinogenic (Wang *et al.* 2015), apoptosis-inducing antibiotic (Zemek *et al.* 1979), and anti-HIV (Lee *et al.* 2011) activities have been reported in lignin-carbohydrate complexes (LCCs). Commodity products with antioxidant or antimicrobial properties, such as sunscreen lotions (Qian *et al.* 2015), biocomposites (Ojagh *et al.* 2011; Domenek *et al.* 2013), and clothes (Kozlowski *et al.* 2008; Zimniewska *et al.* 2008), that use lignin as a natural ingredient have been prepared, and their characterization has shown promising results. The literature describing the biological properties of technical lignins has grown rapidly in the last 10 years. To our knowledge, there are no reviews on the topic of biological activity of technical lignins and LCCs. The aim of this review is to present the most relevant and current research concerning the biological activity of technical lignins, as well as LCCs. The most relevant applications of the biological activity of technical lignins and LCCs are highlighted in this article, including antioxidant, antimicrobial, antimutagenic, anti-inflammatory, antiviral, and other activities that can have positive impacts in the medical, food, and plastic industries.

Chemical Composition of Lignin

Lignin is responsible for the strength and rigid structure of the cell walls in plants. It controls the water conduction and protects the plant against biochemical stresses by inhibiting the enzymatic degradation of other chemical components in it (Boerjan *et al.* 2003). Lignin is an amorphous polymer composed of various phenylpropane units that originate from three aromatic alcohols (monolignols, namely *p*-coumaryl, coniferyl, and sinapyl alcohols) (Lewis and Yamamoto 1990). The aromatic constituents of these alcohols are *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) moieties. During the lignification process, the monolignols are connected by radical coupling reactions to form the lignin polymer network. The main linkages in the lignin polymer are carbon-oxygen and carbon-carbon (β-*O*-4, α-*O*-4, 4-*O*-5, β-5, β-1, 5-5, and β-β linkages) (Buranov and Mazza 2008). The lignin polymer lacks a regular and repetitive order of monomeric units as found in cellulose or protein. The lignin content and its composition varies according to the plant species. Lignin from herbaceous plants contains aromatic units H, G, and S in different proportions; whereas the lignin from woody plants primarily contain G and S units (Lapierre *et al.* 1995; Billa *et al.* 1998). Based on the first proposed structure of lignin by Adler (1977), lignin is recognized as a highly branched polymer with a variety of functional groups, including hydroxyl (aliphatic and aromatic), carboxylic, carbonyl, and methoxyl groups (Adler 1977).

Technical Lignins

Technical lignins are obtained as by-products from the industrial processing of wood, energy crops, or agricultural residues. There are several technical lignins, which obtain their name based in the method of extraction, and they can be classified into two main categories; sulfur-containing lignins and sulfur-free lignin (Lora and Glasser 2002). Sulfur-containing lignins include lignosulfonates and kraft lignin, which are obtained under strongly alkaline treatment of wood. It is estimated that the worldwide production levels of such sulfur-containing lignins are greater than 100,000 tons/year and 1 million tons/year, respectively (Gosselink *et al.* 2004b). The second category comprises sulfur-free lignin, which is obtained mainly from the conversion of lignocellulosic biomass through soda, organosolv, and ionic liquid processes (Ruiz *et al.* 2011). Sulfur-free lignins are an emerging class of lignin obtained from biorefinery processes. Companies around the world such as Green Value (India), CIMV (France), Lignol Innovations (Canada), and Dedini (Brazil) have improved and patented several production processes for sulfur-free lignins (Laurichesse and Averous 2014). Although sulfur-free lignins are obtained in smaller amounts compared to sulfur-containing lignins, the former have important properties that may evolve into industrial-scale value-added products (Gosselink *et al.* 2004b). In the next section, the extraction processes of the sulfur-containing and sulfur-free components of the most important technical lignin are briefly discussed.

SULFUR-CONTAINING LIGNINS

Kraft Lignin

The kraft process is the traditional method to obtain cellulose pulp from biomass using sodium hydroxide and sodium sulfide under strongly alkaline conditions, to cleave the bonds of the wood chemical components (Chakar and Ragauskas 2004). This process produces a large amount of kraft lignin. During kraft cooking of wood, lignin is depolymerized because of the cleavage of aryl ether bonds and degraded into various fragments of different molecular weights, which become soluble in alkali solutions (Chakar and Ragauskas 2004). After cooking, the alkali-dissolved lignin is acidified and concentrated for recovery. The recovered kraft lignin contains several characteristic features, which distinguishes it from native and other technical lignins (Lange *et al.* 2013). One of the main characteristics is the presence of high amounts of condensed chemical structures and the high level of phenolic hydroxyl groups, resulting from the extensive cleavage of β-aryl bonds during the cooking process. Kraft lignin contains sulfur in its chemical structure as a result of the sulphidation (Vishtal and Kraslawski 2011). In addition, kraft lignin presents dark color and it is insoluble in water (Lange *et al.* 2013). Kraft lignin has many applications; however, most are related to the production of low tonnage or pilot-scale products. Mostly kraft lignin is used in low added-value applications or in the production of process steam and energy (Mohan *et al.* 2006). The most relevant characteristics and properties of sulfur-containing and sulfur-free lignins are shown in Table 1.

Lignosulfonates

Lignosulfonates are another class of sulfur-containing lignin obtained from the sulfite chemical pulping process, which is based on the cooking of wood with an aqueous solution of sulfur dioxide (SO_2) and a base (calcium, sodium, magnesium or ammonium) (Doherty *et al.* 2011). Lignosulfonates contain a high amount of sulfur in the form of sulfonate groups present on the aliphatic side chains (Laurichesse and Averous 2014). In addition to sulfonate groups, lignosulfonates also contain other metal contaminants from the chemicals used during pulp production and recovery. The lignosulfonates are highly cross-linked polymers with an approximately 5% sulfur content and is comprised of two types of ionizable groups: sulfonates (pKa ≤ 2) and hydroxyl groups (pKa ~10).

Table 1. Physicochemical Properties of Technical Lignins

	Kraft	**Lignosulfonates**	**Soda**	**Organosolv**	**References**
Solubility	Alkali	Water	Alkali	Organic solvents	(Vishtal and Kraslawski 2011)
Molecular weight (*Mw*)	100 to 3000	20,000 to 50,000	800 to 3,000	500 to 4000	(Laurichesse and Averous 2014; Nevarez *et al.* 2011; Vishtal and Kraslawski 2011)
Polydispersity	2.5 to 3.5	6 to 8	2.5 to 3.5	1.3 to 4.0	(Delmas *et al.* 2011; Fredheim *et al.* 2002)
Impurities	Sulfur	Sulfur	Carbohydrates and ash	Carbohydrates and ash	(Gosselink *et al.* 2004b; Vishtal and Kraslawski 2011)

Lignosulfonates are polyelectrolytes in which the charged groups consist of sulfonic, phenolic hydroxyl, and carboxylic acid groups (Areskogh *et al.* 2010a,b). The lignosulfonates are quite soluble in water and alkali or basic solutions, as well as in highly polar organic solvents. The average molar mass of lignosulfonates is higher than that of kraft lignin, with a broad polydispersity index (Laurichesse and Averous 2014). In terms of commercially available lignin, lignosulfonates represent the most explored technical lignins for several industrial applications, such as binders, dispersing agents, surfactants, adhesives, and as cement additives (Vishtal and Kraslawski 2011).

SULFUR-FREE LIGNINS

Organosolv Lignin

Organosolv lignin is the common name for lignin obtained as a by-product of the fractionation of hardwood, softwood, and herbaceous crop residues, using solvents such as alcohols, organic acids, or mixtures of them (Chum *et al.* 1988; Thring *et al.* 1990; Deng *et al.* 2008). In the organosolv processes, lignin is separated *via* solubilization by the acid-catalyzed cleavage of bonds, such as α-aryl ether and aryl glycerol-β-aryl ether bonds in the lignin macromolecule (Sarkanen 1990). Solubilization makes it possible to obtain a less modified lignin with a chemical structure close to that of native lignin (Duval and Lawoko 2014; Laurichesse and Averous 2014). Organosolv lignins are recovered from the solvent by precipitation, which typically involves adjusting a different parameter, such as concentration, pH, or temperature (Vázquez *et al.* 1997). Organosolv lignins are reportedly the purest form of lignin with the highest quality (El Hage *et al.* 2009; Sannigrahi *et al.* 2010). Interesting properties such as low molecular weight, narrow molecular weight distribution, poor solubility in water, and high phenolic and aliphatic hydroxyl contents have been reported (Lora *et al.* 1989; Delmas *et al.* 2011; Vishtal and Kraslawski 2011; Garcia *et al.* 2012a) for this type of lignin. Other important features of organosolv lignin are its homogeneity, low impurities content, and absence of sulfurous odor (Vallejos *et al.* 2011). All these properties make organosolv lignin an attractive source of natural compounds with the capacity to replace some synthetic compounds.

Soda Lignin

Soda lignin is obtained by treating lignocellulosic materials such as bagasse, sisal, wheat straw, hemp, or kenaf with highly alkaline solutions (typically sodium hydroxide) under conditions similar to kraft pulping, but without inclusion of hydrogen sulfide anions (Doherty *et al.* 2011; Duval and Lawoko 2014). In the soda pulping process, lignin extraction arises due to the hydrolytic cleavage of the native lignin network. Several steps, including acid precipitation, heating, and filtration are used to recover lignin by this process. A relatively, chemically unmodified lignin compared to the other lignin types is obtained. Soda lignin contains no sulfur and a low quantity of hemicellulose, and its properties are quite similar to that of kraft lignin. The absence of sulfur makes it particularly attractive for the preparation of lignin-based materials (Košíková and Gregorová 2005; Wörmeyer *et al.* 2011; Laurichesse and Averous 2014). However, soda lignin can contain high amounts of silicate and nitrogen contents because of the nature of the extraction procedure used (Gosselink *et al.* 2004a,c; Buranov and Mazza 2008; Lora 2008; Wörmeyer *et al.* 2011). The soda processes have been adapted in paper factories for cellulose production from annual plant or agricultural residues. Companies such as Granit SA (Green Value SA – Switzerland) has adapted and enhanced the soda process using the precipitation of lignin from black liquor, by adjusting the pH with various mineral acids.

Antioxidant Properties of Technical Lignins

Antioxidants are molecules that can scavenge or inhibit free radicals present in living systems and foods, preventing oxidation (Gulcin 2012; Salem *et al.* 2014). Antioxidants have significant applications in several industries such as pharmaceuticals, cosmetics, food, plastics, and oil fuel production (Sindhi *et al.* 2013). In these commodities, the commonly used antioxidants include synthetic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG) (Gulcin 2012). Synthetic antioxidants present both beneficial and harmful biological effects at the molecular, cellular, and organ levels (Carocho and Ferreira 2013). However, carcinogenic effects have been observed for synthetic antioxidants when used in high doses; their maximum levels are recommended and controlled by various legislating authorities such as the FDA or/and European Food Safety Authority (EFSA), depending on the final use of the product. In the last century, some researchers have demonstrated that BHA and BHT can cause cytotoxicity and carcinogenesis in laboratory animals, even in low concentrations (Altmann *et al.* 1985a,b, 1986; Grice 1988; Thompson and Moldeus 1988). Additionally, it is recognized that naturally occurring antioxidants are more efficient and safer than some synthetic antioxidants (Faustino *et al.* 2010). These factors have motivated the exploration of renewable resources to obtain natural antioxidants (Gulcin 2012; Ponomarenko *et al.* 2014). Because of their biodegradability and low toxicity, natural antioxidants such as polyphenols have garnered interest as suitable alternatives to synthetic compounds (Malik and Krohnke 2006; Jamshidian *et al.* 2012). Lignin is an aromatic polymer with a complex chemical structure, containing many aromatic rings with hydroxyl and methoxyl functional groups. Due to the presence of these functional groups, oxidation propagation reaction can be terminated through hydrogen donation (Lu *et al.* 1998; Dizhbite *et al.* 2004). Technical lignins are obtained as an abundant waste from the industrial processing of wood, energy crops, or agricultural residues. Technical lignins are being considered as potential antioxidants because their hindered phenolic groups can act as stabilizers in reactions induced by oxygen and its reactive species and condition slowdown of ageing of composites and biological systems (Arshanitsa *et al.* 2013). The most common technical lignins and assays to evaluate their antioxidant activity are shown in Table 2.

Technical lignin shows different physicochemical properties depending on the sources, processing, as well as post-treatments (Sahoo *et al.* 2010). It has been found that different extraction processes can influence the antioxidant properties of lignin, even when it is obtained from the same raw material (Garcia *et al.* 2010). Due to its importance, the effect of the process parameters on the antioxidant potential of lignins has been investigated. Pan *et al.* (2006) analyzed the antioxidant potential of 21 organosolv, ethanol lignin samples from hybrid poplar (*Populus nigra x P. maximowiczii*) under different extraction conditions. They found that the lignins obtained at high temperature using long reaction times, and diluted ethanol showed high antioxidant activity. These lignins were characterized by having more phenolic hydroxyl groups, less aliphatic hydroxyl groups, low molecular weight, and narrow polydispersity. Similar results were obtained by El Hage *et al.* (2012) in ethanol organosolv *Miscanthus x giganteus* lignin, Ugartondo *et al.* (2009), and Ma *et al.* (2013). On the other hand, high molecular weight, high aliphatic hydroxyl group content, heterogeneity, and wide polydispersity (M_w/M_n) were related to negative effects in the antioxidant activity of lignin (Toledano *et al.* 2010a,b). In addition, the presence of carbohydrates can also decrease antioxidant activity (Lawoko *et al.* 2005; Ugartondo *et al.* 2008).

The cytotoxic effects of lignin from bagasse, lignosulfonates, Curan, and steam explosion have also been analyzed (Ugartondo *et al*. 2008), and the half-maximal inhibitory concentration (IC50) values, similar to epicatechin, were found in bagasse lignin (42.3 µg/mL) (Mitjans *et al.* 2004). Cytotoxic assays revealed that these lignins exert cytotoxic effects, but only at very high concentrations (700 to 1200 µg/mL in 3T3 mouse fibroblasts). The relationship between potential cytotoxic properties and the antioxidant capacity of lignins demonstrated that the strongest antioxidant products were also generally the most cytotoxic; nevertheless, the effective antioxidant concentrations are smaller than the cytotoxic concentrations; therefore, the compounds are antioxidants at non-cytotoxic concentrations. Furthermore, the same lignins (bagasse, lignosulfonates, Curan, and steam explosion) are not harmful to eyes or skin (Vinardell *et al.* 2008). Some sugarcane bagasse lignins have proven to be more efficient than BHT (Kaur and Uppal 2015), and even lignins from sisal and abaca have shown radical scavenging above 20%

compared to BHT and tocopherol, the two popular commercial antioxidants used in the food and cosmetic industries (Boeriu *et al.* 2004). The results of these investigations have revealed new perspectives on the potential use of industrial lignin in cosmetics and relevant formulations.

Table 2. Technical Lignins and their Antioxidant Assays

Lignin	**Antioxidant Assay**	**Results**	**References**
Organosolv	$DPPH^{\bullet}$	*RSI* 12.5 to 122	(Pan *et al.* 2006)
Autohydrolysis Soda Organosolv	$DPPH^{\bullet}$	*Reduction (%)* 22 10 to 15 20 to 30	(Garcia *et al.* 2010)
Organosolv	$DPPH^{\bullet}$	*RSI* 0.25 to 0.40	(Vanderghem *et al.* 2011)
Organosolv	ORAC	µmol TE/g 1741.72- 3119.68	(Dong *et al.* 2011)
Organosolv	$DPPH^{\bullet}$	*IC50* (mg/ml) 2.70 to 32.2	(Lu *et al.* 2012b)
Organosolv	Oxygen uptake inhibition (OUI)	*OUI (%)* 58 to 91	(El Hage *et al.* 2012)
Organosolv	$DPPH^{\bullet}$	*IC50* (mg/ml) 0.658	(Lu *et al.* 2012a)
Organosolv	$ABTS^{\bullet +}$	*AOP (%)* 12.72 to 97.96	(Garcia *et al.* 2012b)
Organosolv	$DPPH^{\bullet}$	*RSI* 1.20	(Zhou *et al.* 2012)
Organosolv	$DPPH^{\bullet}$	*RSI* 0.35 to 1.15	(Li *et al.* 2012a)
Organosolv	$DPPH^{\bullet}$	*RSI* 0.25 to 0.40	(Li *et al.* 2012b)
Organosolv°	Oxygen uptake inhibition (OUI)	*OUI (%)* 75 to 82	(Hussin *et al.* 2014)
Organosolv	$DPPH^{\bullet}$ $ABTS^{\bullet +}$ H_2O_2	*IC50 (%)* 80 to 145 2.7 to 3.9 101 to 178	(Aadil *et al.* 2014)

Lignin	Antioxidant Assay	Results	References
Organosolv alkali	DPPH$^{\bullet}$	*RSI* 0.86 to 1.86	(Sun *et al.* 2014a)
Kraft	DPPH$^{\bullet}$ ABTS$^{\bullet+}$ FRAP	*mmol of Trolox* 282 to 511 1485 to 2906 579 to 953	(Sun *et al.* 2014b)
Alkali*	Reducing power	*IC50* (µg) 405.4 to 105.3	(Barapatre *et al.* 2015)
	H_2O_2	*Scavenging (%)* 30.09 to 63.56	
	DPPH$^{\bullet}$	*Scavenging (%)* 37.94 to 71.94	
Kraft Soda Organosolv	Oxygen uptake inhibition (OUI)	*OUI(%)* 55 74 60	(Hussin *et al.* 2015b)
Alkaline	DPPH$^{\bullet}$	*Inhibition (%)* 7.3-12.6	(Kang *et al.* 2015)
Lignosulfonates	FRAP	*EGA (mmol)* 0.5-0.7	
Alkaline	DPPH$^{\bullet}$	*Inhibition (%)* 86.9	(Azadfar *et al.* 2015)
Kraft Soda Organosolv	Oxygen uptake inhibition (OUI)	*OUI (%)* 79 83 77	(Hussin *et al.* 2015a)

Abbreviations: DPPH$^{\bullet}$ 2,2-Diphenyl-1-picrylhydrazyl, ABTS$^{\bullet+}$ 2,2-Azinobis 3-ethylbenzthiazoline-6-sulphonic acid radical scavenging, $O_2^{\bullet-}$ Superoxide anion radical, ORAC Oxygen radical absorbance capacity, OUI Oxygen uptake inhibition, FRAP Ferric reducing antioxidant power, RSI Radical scavenger index, IC50 The concentration of sample required to scavenge DPPH radical by 50%, OAP Antioxidant power, and EGA Equivalent gallic acid. *Bio-modified and unmodified lignin, Organosolv° lignin with autohydrolysis, 2-naphthol, and dilute sulfuric acid pretreatment. NR No reported.

One of the important issues associated with the antioxidant activity of lignin involves the reaction mechanism, which has not been completely known until now. Nevertheless, Perez-Perez *et al.* (2005) proposed three possible mechanisms to explain the antioxidant activity of lignin: (1) lignin acts as a chelating agent, by scavenging metals of Fenton's reagent; (2) lignin acts as a "suicide" antioxidant, accepting the hydroxyl radicals to prevent the action of this radicals on other target molecules; or (3) lignin inhibits the enzymes involved in the metabolic pathways which are capable of generating free radicals (Perez-Perez *et al.* 2005). Many investigations agree with the idea of lignin acting as a scavenger of free radicals due to the presence of phenolic structures in it (Cazacu *et al.* 2013). The hydroxyl groups of phenols can scavenge peroxyl radicals. Moreover, phenols have the capacity to reduce or chelate the divalent ions, necessary for several reactions, and they can also interrupt oxidation reactions by hydrogen atom transfer or by electron transfer with the formation of the phenoxy radical cation, which is rapidly and reversibly deprotonated to form a phenoxy radical (Cazacu *et al.* 2013).

An important issue is the method used to measure the antioxidant activity in lignins. Due to the low solubility of lignins in several solvents, some of these methods or assays have been modified or improved for application to lignin. The most important assays are based on single electron transfer (SET) and hydrogen atom transfer (HAT) reactions (Gulcin 2012). The most reported methods to determine the antioxidant activity of lignins include 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging, oxygen radical absorbance capacity (ORAC), 2,2-azinobis 3-ethylbenzthiazoline-6-sulphonic acid radical ($ABTS^{\bullet+}$) scavenging, superoxide anion radical ($O_2^{\bullet-}$) scavenging, oxygen uptake inhibition, and ferric ion reducing antioxidant power (FRAP) assays (Table 2). The antioxidant assay using the stable radical $DPPH^{\bullet}$ is a fast and easy form of obtaining information about the antioxidant activity of lignin. The evaluation of the radical scavenging activity of BIOLIGNIN™ using three antioxidant assays ($ABTS^{\bullet+}$, $DPPH^{\bullet}$, and $O_2^{\bullet-}$) was reported by Arshanitsa *et al.* (2013).

Fractionation of Lignin to Improve Antioxidant Activity

The antioxidant properties of the different lignins open a variety of fields for their application in the healthcare, cosmetic, and polymer industry. However, due to the high molecular complexity of the lignin, it has been difficult to assign their antioxidant efficiency to specific structural elements (Sakagami *et al.* 2005). Furthermore, the presence of impurities (*e.g.* polysaccharides) may influence the efficiency of the lignins as antioxidants (Dizhbite *et al*. 2004; Pan *et al.* 2006). Fractionation of technical lignins has been investigated as an effective way to reduce the problems related to their antioxidant properties. There are mainly three methods of fractionation of lignin; fractionation by successive extraction with organic solvents, differential precipitation, and ultrafiltration (Toledano *et al.* 2010a,b; dos Santos *et al.* 2014).

Fractionation by successive extraction with organic solvents allows separating fractions of lignin according to the solubility in the solvent of extraction. Common solvents reported to fractionate lignin include alcohols, organic acids, or admixtures of them (Wang *et al.* 2010; Boeriu *et al.* 2014). Fractionation by successive extraction with organic solvents allows obtaining low molecular weight lignin (LMWL) fractions dissolved in the solvents; while the lignin with higher molecular weight is enriched into the residue (Ropponen *et al.* 2011). Fractionation by successive extraction with organic solvents reduces the heterogeneity of lignin because different parts of the lignin molecule have different hydrogen bonding capacity with different organic solvents (Wang *et al.* 2010). Schuerch (1952) mentioned that LMWL fragments are dissolved in organic solvents and have weaker hydrogen-bonding capacities than the higher-molecular weight lignin fragments. Boeriu *et al*. (2014) stated that LMWLs are less condensed and have lower aliphatic hydroxyl content than parent lignins. Li *et al*. (2012c) investigated the effect of the fractionation on the antioxidant capacity of bamboo organosolv lignin in several solvents (ether (F1), ethyl acetate (F2), methanol (F3), acetone (F4), and dioxane/water (F5)). They found that the antioxidant capacity of the lignin fraction decreased when the dissolving capacity of solvent increased. F1 showed the highest radical scavenger index (RSI) and it was related to the high content of phenolic hydroxyl groups, which were essential for the formation of large amounts of phenoxyl radicals. In addition, the high amount of the methoxyl group present in F1 stabilized the phenoxyl radicals formed. The advantages and disadvantages of the most important fractionation methods of lignins are shown in Table 3.

Differential precipitation procedures separate lignin fractions by gradual acidification, producing different precipitates according to the pH (Sun and Tomkinson 2001; Mussatto *et al.* 2007). This is a common method used in laboratories or industrial plants because the simple addition of a strong mineral acid (*e.g.* sulphuric or hydrochloric acid) is enough to reach the objective. However, colloids formation during precipitation can complicate the subsequent process of separation (Toledano *et al.* 2010a). Differential precipitation of technical lignin has been widely investigated (Sun *et al.* 1999; Sun and Tomkinson 2001; Mussatto *et al.* 2007; Garcia *et al.* 2009). Sun *et al.* (2001) obtained 93.6% recovery of lignin by precipitation at pH 7, and it was relatively free of non-lignin matter. The yield and purity of lignin fractions can be increased by decreasing the pH (Sun *et al.* 1999). For example, fractions of soda lignin with similar physicochemical characteristics to commercial alkali lignin were obtained at pH values between 0.72 and 2.57 (Garcia *et al*. 2009). Regarding antioxidant activity of the fractions of lignin obtained using selective precipitation, Ma *et al.* (2013) stated that the neutralization of the phenolic hydroxyl groups in kraft lignin occurs in the pH range of 10.5 to 11. Therefore, the lignin fraction precipitated at pH values higher than 10.5 contains few phenolic groups. They concluded that the lignin fractions precipitated at pH 2 can possess the best radical scavenging ability. This is related to the number of phenolic hydroxyl groups and the lower molecular weight of the lignin that can only be precipitated at very low pH.

Ultrafiltration using ceramic membranes of different cut-offs was used to obtain lignin fractions from the black liquor. Ultrafiltration is an easy and reagent-free technique to obtain lignin with defined molecular weight distributions without the addition of reagents. Furthermore, the use ultrafiltration does not require any change of the pH or the temperature of the lignin solution (Colyar *et al.* 2008; Jönsson *et al.* 2008). Toledano *et al.* (2010) utilized ultrafiltration to analyze the chemical properties and behavior of black liquor of *Miscanthus sinensis* by using different cut-offs membranes. LMWL fractions were obtained in the fractions separated by 5 and 15 kDa cut-offs membranes, and such fractions did not have significant differences in their chemical composition. Toledano *et al*. (2010) demonstrated that ultrafiltrated fractions of lignin are less contaminated than fractions obtained by selective precipitation. Garcia *et al.* (2010) mentioned that permeated lignin produced by the ultrafiltration method will possess a good antioxidant activity in comparison to the initial rough lignin. An ultrafiltration process significantly improves the antioxidant capacity of lignins by decreasing the polydispersity and improving the solubility of lignin. Recently, Hussin *et al.* (2015a) found that lignin with low average molecular weight, high phenolic OH content (especially the phenolic S units), and better solubility can be produced by using ultrafiltration. Furthermore, antioxidant assays (oxygen uptake and reducing power) revealed that phenolic OH and ortho-methoxyl are responsible for stabilizing the radical formed. In addition, Hussin *et al.* (2015a) proposed several possible applications of ultrafiltrated lignin.

The literature describing the technical lignins as raw materials to produce naturally occurring antioxidants has increased in the last 10 years (Dong *et al.* 2011; Vanderghem *et al.* 2011; Zhou *et al.* 2012; Hussin *et al.* 2015a; Kang *et al.* 2015). Lignin provides a natural and relatively safe source of antioxidants compared to the costly and less efficient synthetic antioxidants (*e.g.*, BHT) (Faustino *et al.* 2010). The chemical complexity of lignins remains the barrier to expand their use as antioxidants, but the modulation of suitable lignin structures (by considering its solubility, molecular weight, phenolic OH content) can overcome the problems related to their limited usage in industrial sectors. Fractionation methods such as extraction in organic solvents, differential precipitation, and ultrafiltration can be used to decrease the complexity of technical lignins and to improve their antioxidant properties (Garcia *et al.* 2010; Arshanitsa *et al.* 2013; Ma *et al.* 2013).

Table 3. Advantages and Disadvantages of the Fractionation Methods of Technical Lignins

Fractionation processes	Advantages	Disadvantages
Fractionation with organic solvents	Fractions with MWD and component composition uniformity Improve the solubility	Use chemical reagents High production costs
Differential precipitation	Fractions with some defined MWD Low energy consuming	Use chemical reagents Contaminated by LCC Colloids formation during precipitation
Ultrafiltration	Fractions with defined MWD and low polydispersity Remotion of impurities Improved solubility Free-reagent treatment	High energy consuming Low yield of extraction

Abbreviations: MWD Molecular weight distribution, LCC Lignin-complex carbohydrates.

Antimicrobial Properties of Lignin

Lignin is a major source of natural antimicrobial compounds. It has been mentioned in early research reports that wood lignin is a phenolic polymeric-compound containing 11 phenolic monomeric fragments with a high content of *p*-coumaric and ferulic acids (Jung and Fahey 1983; Zemek *et al.* 1987). Microorganisms such as *E. coli*, *S. cerevisiae*, *B. licheniformis*, and *A. niger* can be inhibited by fragments of lignin (Zemek *et al.* 1979). This inhibitory effect was explained by the presence of a double bond in the C_α=C_β position of the side chain and a methyl group in the γ-position. In general, phenolic fragments containing a C-C double bond in the α- and β-positions of the side chain and a methyl group in the γ-position are more inhibitory than phenolic fragments with functional groups containing oxygen (–OH, –CO, COOH) in the side chain. The antimicrobial properties of various softwood and hardwood lignins as well as the modified-oxidized lignin samples (organosolv, sulfite, and kraft lignins) have been tested on a series of yeasts (Sláviková and Košíková 1994), demonstrating that the antimicrobial effect of oxidized lignins was lower than that of unmodified samples, except on *Sporobolomyces roseus*. Unmodified lignins showed an inhibitory effect against *Candida tropicalis, Trichosporon cutaneum,* and *Candida albicans*. This difference in antimicrobial activity was attributed to the different genetic origin of lignin sample and its processing method. Both soluble and insoluble fractions of kraft lignins demonstrated an inhibitory effect against phytopathogenic bacteria (Dizhbite *et al.* 2004). Black liquors from bagasse and cotton stalks were effective against gram-positive bacteria (*B. subtilis* and *B. mycoides*), but gram-negative bacteria (*E. coli*) and filamentous fungi (*A. niger*) were not affected. Additionally, the antibacterial activity of *p*-hydroxycinnamaldehyde synthesized from the reduction reaction of *p*-hydroxycinnamic acid was reported. The compound *p*-hydroxycinnamaldehyde has a broad spectrum of antibacterial activity against not only gram-negative but also gram-positive bacteria (Leem *et al.* 1999). Tests performed on various lignin preparations have shown that the antimicrobial activity depends on the lignin origin, extraction method (Sláviková and Košíková 1994), chemical structure (Jung and Fahey 1983), concentration in the cultivation medium (Nelson *et al.* 1994), and microorganism strain (Nada *et al.* 1989). The mechanism of antibacterial activity of lignin varies in relation to the nature of phenolic compounds (Barber *et al.* 2000). In general, the polyphenolic compounds of lignin cause cell membrane damage and lysis of bacteria with subsequent release of their cell contents.

The antibacterial activities of hemp and other fiber plants such as jute, flax, kenaf, sisal, and bamboo were recently reviewed (Mahmood *et al.* 2013). The antibacterial character of those fibers was associated with the presence of cannabinoids, alkaloids, and phenolic compounds contained in lignin samples. Recent research has demonstrated that the origin of the antibacterial property of bamboo (*Phyllostachys pubescens*) is located in lignin (aromatic and phenolic functional groups) and not in hemicellulose or the other water-soluble chemical components of biomass as previously reported (Afrin *et al.* 2012). Phenolic compounds such as isoeugenol, butyl *p*-hydroxybenzoate, *p*-coumaric acid, and ferulic acid can provide antimicrobial activity in kraft pulping by bio-modification (Pei *et al.* 2012). Furthermore, the antimicrobial activity of lignin extracted from the residues of corn stover in bio-ethanol production was investigated (Dong *et al.* 2011); lignin extracts exhibited antimicrobial activity against gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) and yeast (*Candida lipolytica*), but not gram-negative bacteria (*Escherichia coli* O157: H7 and *Salmonella enteritidis*) or bacteriophage MS2. The lack of activity of lignin against gram-negative bacteria is consistent with previous studies (Nada *et al.* 1989); in addition, different extraction conditions (temperature and residue/solvent ratio) affect the antimicrobial activity of lignin extracts. Some researchers have reported a correlation between the antioxidant property of lignin and their antibacterial properties. Dizhbite *et al.* (2004) assumed a connection of the antibacterial effect of kraft lignin with radical scavenging activity of soluble fractions, and Dong *et al.* (2011) claimed that the antimicrobial activities of lignin extracts from corn stover were consistent with their antioxidant activities.

Antigenotoxic and Antimutagenic Properties of Lignin

Genotoxic compounds are known for their ability to damage both DNA and cellular components that are related to the functionality and behavior of chromosomes within the cell, and these damages may be mutagenic or carcinogenic. The antigenotoxic activities of different lignins have been demonstrated using *in vitro* and *ex vivo* experiments. This research demonstrated that the lignins are not toxic, and they have the potential to protect living organisms against damage from different genotoxicants (Mikulášová and Košíková 2003; Košíková and Lábaj 2009; Košíková and Lábaj 2010). The antigenotoxic activity of lignins has been demonstrated against different genotoxic compounds including N-nitrosodiethylamine (NDA), 1,2-dibromo-3-chloropropane (DBCP), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), ofloxacin, 4-nitroquinoline-N-oxide (4NQO), 3-5-nitro-2-furyl (5NFAA), 2-nitrofluorene (2NF), and hydrogen peroxide (H_2O_2) (Košíková *et al.* 1990; Horváthová *et al.* 1999; Slameňová *et al.* 1999; Ebringer *et al.* 1999; Krizkova *et al.* 2000; Mikulášová and Košíková 2002; Mikulášová and Košíková 2003; Lábaj *et al.* 2003; Košíková *et al.* 2008).

Both unmodified and biologically modified lignins have been examined as chemopreventive agents in cancer development. These lignins exert high DNA protection towards oxidative damage through the scavenging of OH radicals and reduction of the alkylating activity of MNNG (Košíková *et al.* 1990, 2002, 2006, 2010). This reduction, which causes instability in N-glucosyl bonds with DNA, may correlate with the lignin adsorption affinity toward N-nitroso compounds (Mikulášová and Košíková 2002). The antimutagenic activity of different types of lignin has been reported, including oxidized, reduced, and unmodified lignins against UV irradiation in the flagellate *Euglena gracilis* (Belicová *et al.* 2000). This antimutagenic activity decreased in the order oxidized > reduced > unmodified lignins. The effectiveness of oxidized lignin has been attributed to the increased oxygen content in an alkaline solution treatment applied to it, which creates new carbonyl groups and *p*-quinones through side chain displacement to yield lignin derivatives, and consequently increases the antimutagenic activity.

Several researchers have demonstrated that sulfur-free lignin biopolymer reduced the amount of DNA strand breaks in $H_2O_2^-$ and MNNG-treated mammalian cells (Horváthová *et al.* 1999; Slameňová *et al.* 1999, 2000; Košíková *et al.* 2002). Moreover, Krizkova *et al.* (2000) demonstrated that the production of the superoxide anion radical ($O_2^{\bullet -}$) by ofloxacin is efficiently eliminated at a lignin concentration of 500 µg/mL. Slameňová *et al.* (2006) reported that the biopolymer lignin at a concentration of 3 mg/mL can protect human cells against the genotoxic effects of 3'-azido-3'-dideoxythymidine (AZT), a known anti-HIV drug. Ebringer *et al.* (1999) mentioned that mutagens may generate some oxidant species and subsequently free radicals or they may interact with lignin itself, which acts as an antioxidant. Specific techniques such as the comet assay have demonstrated that antimutagenic effects of lignin against genotoxic compounds are associated with both adsorptive and antioxidative actions (Lábaj *et al.* 2003; Mikulášová and Košíková 2003).

The results of the aforementioned research demonstrate the potential of lignin biopolymers as antimutagenic agents in chemoprevention. Because of the drawbacks of synthetic compounds in humans, natural compounds are receiving more attention for potential application in biomedicine (Hertog *et al.* 1993). Lignin biopolymer has shown the capability to reduce genotoxic activity by scavenging reactive oxygen species. The antigenotoxic and antimutagenic effects of different lignins indicate their prospective application as natural agents for the prevention of carcinogenesis and other diseases instead of compounds prepared by organic synthesis. Nevertheless, more research is needed to establish the effect that these biopolymers exert in *in vivo* studies because their pharmacokinetics has not yet been determined.

Lignin-Carbohydrate Complexes and Lignin-related Compounds with Biological Activity

Lignin-carbohydrate complexes (LCCs), LMWL, and monomers degraded from lignin polymer also possess some biological properties such as antioxidant, anti-inflammatory, antihypertensive, and antispasmodic properties (Bjørsvik and Liguori 2002; Sato *et al.* 2009; Lee *et al.* 2012; Qiu *et al.* 2012; Yoshioka *et al.* 2012) that can benefit human health. Structural variants of lignin (monomers and oligomers) have been recognized as functional molecules to mimic low molecular weight heparins (synthetic anticoagulation agents) (Monien *et al.* 2006; Henry *et al.* 2007, 2009). An oligomer of lignin was identified as potent inhibitors of coagulation proteinases *in vitro* and *in vivo*. Sulfated LMWL present powerful leads for the discovery of selective agents that target fXIa (validated drug targets) (Henry *et al.* 2012). In this sense, the chemo-enzymatic synthesis of novel LMWLs from 4-hydroxycinnamic acid monomers (caffeic acid, ferulic acid, and sinapic acid), which can serve as potential anti-emphysema agents, was reported for the first time by Saluja *et al.* (2013). These novel unsulfonated and sulfonated LMWL exhibited potent triple inhibitory activity against elastase, oxidation, and inflammation, the three major pathogenic mechanisms known to cause emphysema (Saluja *et al.* 2013). Differences in their molar inhibitory efficiency were detected depending on the nature of the monomeric structure and the absence or presence of sulfur in it. However, the sulfonated caffeic acid-based moiety (named CDSO3) displayed the most efficient triple-action inhibitory effect.

Moreover, fractions from cultured *Lentinula edodes* mycelia (LEM) have shown potential as hepatoprotective agents (Yoshioka *et al.* 2012). The strongest hepatocyte protective activity has been observed in the fractions composed of lignins and xylan-like polysaccharides; however, the hepatocyte protective activity can be completely attributed to the lignin. Furthermore, the antiviral effect of lignin from LEM has been demonstrated. These lignins have been tested, are minimally cytotoxic (IC_{50} 5 µg/mL) and strongly inhibited the entry of hepatitis C virus (HCV) into cells (Matsuhisa *et al.* 2015). The lignin present in LCC has the ability to inhibit the entry of HIV and herpes simplex virus, and it also has a synergistic effect when combined with vitamin C (Sakagami *et al.* 2005, 2010; Lee *et al.* 2011; Qiu *et al.* 2012).

A complete solubilization and sterilization of LCCs can be achieved by autoclaving under mildly alkaline conditions, and this process does not affect the biological activities of these compounds (Sakagami *et al.* 2011). For example, cacao mass and cacao husk LCCs have demonstrated reproducibility in anti-HIV assays after solubilization and sterilization treatments. Moreover, cacao mass LCCs have several unique biological properties distinct from lipoproteins, namely higher anti-HIV activity. In addition, the cacao husk lignin fractions possess anti-influenza virus activity, but no antibacterial activity. The cacao husk lignin fractions synergistically enhanced the superoxide anion and hydroxyl radical scavenging activity of vitamin C (Sakagami *et al.* 2008). Furthermore, anti-herpes activities of LCC from *Prunella vulgaris* L. have been demonstrated (Zhang *et al.* 2007).

Another biological activity was recently reported; two water-soluble lignin metabolites (designated as IOW-S-1 and IOW-S-2) from a hot-water extract of *Inonotus obliquus* were demonstrated to be antiproliferative agents (Wang *et al.* 2015). The chemical characteristics of these metabolites include molecular weights between 25 and 40 kDa composed predominantly by hydrophobic lignin highly water-soluble, along with ~20% carbohydrates. Cytotoxic tests revealed that lignin derivatives can induce cell apoptosis. In addition, these water-soluble lignin metabolites inhibited the activation of the nuclear transcription factor NF-kB in cancer cells, an especially important factor in modulating the expression of immunoregulatory genes relevant in critical illness, inflammatory diseases, apoptosis, cancer, immune diseases, septic shock, and viral infection (Sun and Andersson 2002). The LMWL obtained from the waste of the paper industry and biorefineries, and also LCC obtained from plants provide a promising future in the fight against viral infections such as HIV, HCV, liver diseases, and other related illnesses.

Applications of Technical Lignins

One of the main applications of technical lignin involves the development of active packaging films, composites, and micro- and nanostructured lignin-based materials (Duval and Lawoko 2014). The effects of the incorporation of lignin on the mechanical, thermal, barrier, and other properties of organic and inorganic composites have been extensively studied in the last 30 years (Baumberger *et al.* 1997; Kadla and Kubo 2003; Spiridon *et al.* 2011; Chantapet *et al.* 2013). However, in the field of lignin-based biomaterials, the evaluation of the antioxidant and antimicrobial activity is relatively a new topic aimed at the preparation of active food packaging. Composites of fish skin gelatin/lignosulfonates and commercial fish gelatin/sulfur-free lignin powders have been prepared and characterized as an alternative in food packaging applications (Núñez-Flores *et al.* 2012; Núñez-Flores *et al.* 2013a). Both lignosulfonates and sulfur-free lignin exhibit cytotoxic effects, but only at very high concentrations. A relationship between cytotoxic potential and antioxidant activity revealed that the effective antioxidant concentration is smaller than the cytotoxic concentration. These results were comparable to those found in fish gelatin films enriched with borage extract (Gómez-Estaca *et al.* 2009). Finally, a clear antimicrobial effect could not be observed in fish gelatin/lignin films, the only notable antimicrobial finding being some activity against yeasts.

Ojagh *et al.* (2011) reported the preparation and characterization of fish gelatin-lignin films to be used as packaging for salmon fillets. The incorporation of lignin improved the quality of salmon fillets by reducing the amount of protein carbonyl groups formed in Atlantic salmon muscle immediately after high-pressure processing. Moreover, the incorporation of lignin decreased lipid oxidation in advanced stages of cold storage (Ojagh *et al.* 2011). Additionally, the capacity of gelatin-lignosulfonate films for preserving sardine fillets during chilled storage in combination with high-pressure treatment has been investigated (Núñez-Flores *et al.* 2013b). The incorporation of lignosulfonates increased the antioxidant properties (ABTS radical scavenging and ferric ion reduction) of gelatin films, which remained practically unaltered during four weeks of storage at room temperature. The gelatin-lignin films conferred stability during the storage of chilled sardines, especially in combination with high-pressure treatment. The results obtained by Ojagh *et al.* (2011) and Núñez-Flores *et al.* (2013b) are promising for fish meat preservation.

The antioxidant properties of composite films prepared from durum wheat starch and alcohol-soluble lignin (ASL) were reported by Espinoza-Acosta *et al.* (2015). The composites obtained exhibited antioxidant activity against the known free radical (2,2-diphenyl-1-picrylhydrazyl [DPPH$^{\bullet}$]), which increased as the ASL content increased (Espinoza-Acosta *et al.* 2015). In addition, the ASL fraction improved both the mechanical and thermal properties of starch-lignin films. Moreover, Domenek *et al.* (2013) reported the preparation of PLA-kraft lignin films by a two-step twin-screw extrusion and thermo-compression process. The increase in the generation of free phenolic monomers as a consequence of thermal treatments positively affected the antioxidant activity of the PLA-lignin films.

The fabrication of implantable biomaterials that are biocompatible and resistant to microbial colonization is currently being investigated. Based on this, biofilms of hydroxyapatite (HA) or silver (Ag)-doped HA combined with organosolv lignin (Lig) (Ag: HA-Lig) assembled onto TiO_2/Ti were prepared and subjected to biological and microbial assays. The incorporation of lignin and/or Ag into TiO_2/Ti had a positive effect on the inhibition of *S. aureus*, *P. aeruginosa*, and *C. famata* 30; moreover, these biomaterials exhibited low cytotoxicity towards human mesenchymal stem cells (Jankovic *et al.* 2015). Lignin has also been incorporated by electrophoretic deposition and electrodeposited on titanium; the resulting materials obtained were non-toxic, and in this case, lignin was able to provide protection against titanium corrosion (Eraković *et al.* 2012; Eraković *et al.* 2013). Therefore, lignin as a natural and abundant biopolymer appears to be a good candidate for the fabrication of implantable biomaterials with important properties such as biocompatibility and resistance against microorganism attack.

Moreover, nanoscale materials such as micro- and nanoparticles (Ge *et al.* 2014; Stewart *et al.* 2014), nanofibers (Kadla *et al.* 2009; Dallmeyer *et al.* 2010), and nanospheres of lignin (Yearla and Padmasree 2015) are being widely investigated. The importance of obtaining nanoscale lignin materials is that decreasing the particle size increases the surface-to-volume ratio, which can also improve the solubility and dissolution of poorly water-soluble lignin (*e.g.,* organosolv lignin) and hence augment its bioavailability. Nanoscale lignin with a mean particle size of ~0.144 µm has successfully been prepared using a supercritical anti-solvent (SAS) process (Lu *et al.* 2012b). The size reduction of lignin to the nanoscale improved its solubility and, at the same time, enhanced its antioxidant parameters, such as $DPPH^{\bullet}$ radical scavenging activity, superoxide radical scavenging activity, and reducing power. The nanoscale lignin acted as a better antioxidant than the non-nanoscale lignin (Lu *et al.* 2012b). Ge *et al.* (2014) obtained nanoparticles from alkaline lignin through a simple solution-precipitation method using an alkaline solution and an ethylene glycol solvent. Higher antioxidant activity was observed in lignin precipitated with the alkaline solution, which had a smaller particle size (278 nm), more phenolic OH groups, and lower molecular weight than lignin precipitated with ethylene glycol. Additionally, the antioxidant activity was related to a faster diffusion of the smaller particles in the antioxidant activity assay (Ge *et al.* 2014). Contrary to the SAS process, the simple solution-precipitation method was reported as a low-cost, fast, and feasible method for the large-scale production of lignin nanoparticles.

In addition to antioxidant properties, lignin nanoparticles have exhibited UV protectant properties. Lignin nanoparticles with a spherical shape and a mean size of 80 to 104 nm were prepared by the nano-precipitation method. Analysis of the radical scavenging activity revealed that the nanoparticles possessed higher antioxidant activity compared to unmodified lignin. Additionally, the nanoparticles had UV protectant properties, which were validated by monitoring the survival rates of *Escherichia coli* upon UV-induced mortality (Yearla and Padmasree 2015). Nanoscale lignin materials have better properties such as solubility, antioxidant activity, and UV protection compared to bulk lignin polymers. Micro- or nano-structures of different technical lignins can lead to promising applications in the cosmetic, pharmaceutical, and food industries.

On the other hand, the stabilizing effect against UV degradation or thermo-oxidation of lignin when it is incorporated into polymer such as polyethylene (Alexy *et al.* 2000), polypropylene (Pouteau *et al.* 2003; Pouteau *et al.* 2004; Gregorova *et al.* 2007), polystyrene (Pucciariello *et al.* 2004) and rubber (De Paoli and Furlan 1985; Gregorová *et al.* 2006), have been demonstrated. Lignin preparations at a concentration of 1 to 2 wt% exhibited the ability to act as processing stabilizers and antioxidants during the thermal aging of polypropylene films (Kosikova and Labaj 2009). Nanoparticles combining chitosan and lignosulfonates were developed for the first time for cosmetic and biomedical applications (Kim *et al.* 2013); this combination produced nanoparticles with high stability to lysozyme degradation, biocompatibility with human cells, and antimicrobial activity against *E. coli* and *B. subtilis*. Nanoparticles based on modified lignins with biocidal properties were reported by Popa *et al.* (2011). These nanoparticles were able to ensure high biological wood stability and improve wood resistance to biodegradation (Ungureanu *et al.* 2008; Gîlcă and Popa 2013).

Ponomarenko *et al.* (2014) reported that fractions of LignoBoost kraft lignin are an excellent source of antioxidants for different products/materials such as polyurethane (PU), elastomers, and rapeseed oil. Qian *et al.* (2015) prepared and evaluated the performance of a broad-spectrum sunblock by adding lignin into several commercial sunscreen lotions; this incorporation enhanced the sunscreen performance of commercial sunscreen lotions at unexpected levels. Although the underlying mechanism remains to be elucidated, it was attributed to a synergistic effect between lignin and other active ingredients in the sunscreen, as well as the antioxidant activity of lignin (Qian *et al.* 2015). In the textile industry, the development of highly functional clothing with additional properties such as UV protection, antistatic behavior, and antibacterial activity is the result of the continuous increase in customers' requirements. Nanoscale lignin has been incorporated in linen fabrics (Kozlowski *et al.* 2008; Zimniewska *et al.* 2008). The benefits obtained by adding nanoscale lignin include improvement in washing resistance, protection against UV rays, and antibacterial properties. Additionally, the incorporation of lignin does not change the color of clothes, even though the lignin is originally dark (Zimniewska *et al.* 2012).

SUMMARY

This review covers a significant amount of literature and recent research regarding the biological activities of technical lignins and lignin-carbohydrate complexes, and their potential applications in important fields such as food, medicine, pharmaceuticals, polymers, and cosmetics. However, more research on the potential applications of lignins must be considered in the cosmetic and pharmaceutical industries because these applications must be completely harmless to humans. Technical lignins or their fractions have been used as natural antioxidant agents to avoid photo- or thermo-oxidation of synthetic polymeric materials. The capacity of sulfur-containing and sulfur-free lignins to act as antioxidants (in bulk polymer, fractions, or nano-sized materials), obtained using several extraction methods has been extensively demonstrated. Antibacterial activity is another important property of technical lignin. The activity of some bacteria (gram-negative and gram-positive) and fungi can be inhibited by lignin. The cytotoxic studies highlighted in this article demonstrate the safety of lignin and lignin-carbohydrate complexes in animals. All these properties are being applied in the manufacture of commodity products such as UV protective lotions, cosmetics, clothes, and packaging materials.

Technical lignins are an abundant but low-value by-product of wood pulp and bioethanol production, making them inexpensive and sustainable. The use of lignin in foods has been underestimated. Until today, there have been few investigations on the use of technical lignin as a food-grade ingredient in food; however, the studies that have been performed on animals have shown promising results. Nevertheless, for human consumption, the cytotoxic effects of food ingredients based on lignin must be carefully studied. In addition to safety, the functionality and consumer acceptance of lignin must also be studied.

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