

CHARACTERIZATION AND COMPARISON BY UV SPECTROSCOPY OF PRECIPITATED LIGNINS AND COMMERCIAL LIGNOSULFONATES

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Five precipitated lignins and nine commercial lignosulfonates were investigated in this study. Lignins were characterized by elemental analysis and as to ash content. To determine the amount of free phenolic groups in isolated precipitated and commercial lignosulfonates, ionization difference UV spectroscopy was used. The objective of this study was to examine the UV-Vis characteristics of precipitated and commercial lignin preparations in an effort to evidence their similarities and dissimilarities. Based on the experimental measurements, significant differences between the described lignins and lignosulfonates were identified. It was found that kraft lignin had a higher content of total amount of phenolic hydroxyl groups than lignin with straw (hemp and flax), isolated for modified alkaline anthraquinone cooking with different acids and commercial lignosulfonates. Moreover, it was confirmed that the content of non-conjugated and conjugated phenolic hydroxyl groups, as well as their total amount in the preparations depended on the method, raw material, and experimental conditions used in material processing.

Keywords: lignin, lignosulfonates, UV spectroscopy

INTRODUCTION

The worldwide increase in the price and cost of petroleum and coal has enhanced the interest in alternative sources of raw materials and fuels. Lignin is a complex phenolic polymer found in biomass feedstocks and biomass derived products. Nature produces a vast amount of 150-170 billion metric tons of biomass per year by photosynthesis, 20% of which can be assigned to the class of amorphous polymer consisting of methoxylated phenylpropane structures.¹ Lignin is often defined from the compositional standpoint as a polymer derived from three hydroxycinnamyl alcohols by a dehydrogenating polymerization involving radical coupling.² Each of these monolignols results in a different type of lignin units called p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S).^{3,4} Lignin composition varies in different groups of vascular plants, G, GS, and HGS lignin being characteristic of softwoods (woody gymnosperms), hardwoods (woody angiosperms), and graminaceous plants (non-woody angiosperms), respectively.⁵ The structural

differences between isolated lignins have been investigated using UV-Vis, FTIR, and NMR spectroscopies. The UV spectrum is one of the most characteristic features of a compound. Absorption bands in the lignin spectra are potentially assignable to a variety of groups or compounds. Lignins contain several functional chemical groups, such as hydroxyl (phenolic or alcoholic), methoxyl, carbonyl and carboxyl, in various amounts, depending on their origin and the applied isolation process.^{6,7} The distinct network structure, as well as the presence of the various chemical substituents, confers unique functional properties to lignins.⁸ The spectral measurement of model lignin compounds with the known composition and lignin derivatives provides a method for determination of lignin functional groups in the derivatives.⁹ In particular, the quantitative analysis of phenolic groups is very important. In recent years, UV techniques have been used to determine phenolic groups in lignin. The method is based on the findings that

phenolic groups in non-ionized (i.e. in neutral media) and ionized (i.e. in alkaline media) forms differ in electronic absorption spectra. This difference can be used to estimate the ratio of various phenolic groups.

It has been found that, due to the presence of phenolic groups, lignin compounds exhibit significant antioxidant activity. Such an activity of this natural product was identified for various synthetic polymers, rubber, and foodstuff.¹⁰⁻¹⁴ This activity has been correlated mainly with the total amount (concentration) of phenolic groups, the attempts to characterize and ascribe the activity to individual groups are very rare. Knowledge of the concentration and/or ratio of the individual groups may be used to optimize the antioxidant activity of lignin itself and its derivatives. This is why, in this work the concentration of individual phenolic groups was determined.

The present study aimed at obtaining data on UV characterization of isolated precipitated and commercial lignins and lignosulfonates from the viewpoint of their composition, and based on their comparison to evidence their similarities and dissimilarities.

EXPERIMENTAL

Characterization of liquor, lignin and lignosulfonates

Black liquor (modified alkaline anthraquinone cooking) characterization

The annual plants (hemp and flax) used for obtaining black liquor were kindly supplied by OP Papirna Ltd. (Olsany, Czech Republic). The black liquor obtained had the following characteristics: pH of 12.9 ± 0.3 (determined by a digital Jenway Model 3510 pH-meter), density 1.242 g/mL (determined by measuring the weight of a known volume of the black liquor).

Black liquor (kraft cooking) characterization

Kraft black liquor was kindly supplied by Bukoza Holding Inc. (Hencovce, Slovak Republic). The black liquor had the following characteristics: pH of 12.8 ± 0.4 (determined by a digital Jenway Model 3510 pH-meter), density of 1.358 g/mL (determined by measuring the weight of a known volume of the black liquor).

Commercial lignosulfonates

Borresperse N, Borremet Ca 120, Vanisperse CB were purchased from Borregard LignoTech, Marasperse N-22 from Daishowa Chemical Inc., Orzan S from ITT Rayonier Inc., DP-02, DP-03 from

Biotech, Sulfonated Kraft lignin Westvaco, Maratan SN from American Can Company.

Lignin recovery from black liquor

The precipitation of lignin from black liquor was initially studied as a single step process in which dilute acid solution (5 wt%) was added to the black liquor with the pH adjusted to the desired value. 100 mL of the black liquor was treated with different amounts of diluted sulphuric acid (or HCl, HNO₃, CH₃COOH) to obtain a final pH value 3 (for CH₃COOH pH = 4.3). After complete precipitation, the content of each flask was filtered through pre-weighed oven-dry filter paper, using a vacuum filtration unit. The precipitated lignin was twice washed with hot water to remove impurities. The lignin was then dried at 25 °C for 24 hours, using lyophilisation equipment (LYOVAC TG) up to reaching a constant weight.

Elemental analysis

Total nitrogen (N), carbon (C), hydrogen (H), and sulphur (S) contents in all samples were determined by dry combustion using a Vario Macro Cube C/H/N/S-analyser (Elementar, Hanau, Germany). Two replicates were measured and the mean standard errors were 0.54% for C, 0.04% for N, 0.41% H and 0.84% for S.

Higher heating value

Higher heating value (HHV) was determined by a FTT Calorimetric Bomb using a slightly modified standard method.¹⁵⁻¹⁷ Details are given elsewhere.¹⁸

UV spectroscopy

After being dried overnight at 80 °C, a precisely weighed amount (about 5 mg) of the lignin was dissolved in 5 mL of dioxane and 5 mL of 0.2 M NaOH. Some of the solutions were not quite clear and these were filtered using a 0.45 µm PVDF membrane filter. From each lignin solution, 2 mL was further diluted to 25 mL using either a pH = 6 buffer solution (citrate – NaOH, Merck), or 0.2 M NaOH solution.¹⁹ This gave each solution a final concentration of lignin of about 0.04 g/L. UV-Vis spectra were recorded on a CECIL spectrophotometer in the absorption region 200-450 nm, scan speed 5 nm/s and resolution 1 nm. The lignin solution with the pH = 6 was used as a reference and the alkaline solutions measured against it. From the difference spectra, the absorbance values at 300 and 350 nm, measured against the solution containing 0.2 M NaOH were recorded. According to an original work of Gartner *et al.*,¹⁹ there exist six structural types of phenolic structures (Fig. 1). Maxima at 300 nm and 350-360 nm are assigned to unconjugated phenolic structures (I and III) and that at 350-370 nm to conjugated structures (II and IV). According to Zakis *et al.*,²⁰ the maximum at 360 nm is attributed only to IIa and IVa types of phenolic structures in lignin.

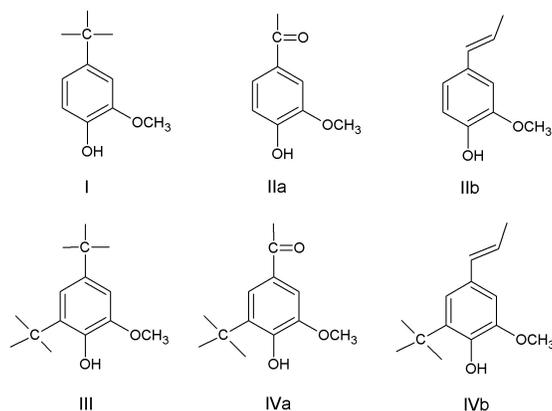


Figure 1: Type of phenolic structures determined by the UV method^{19,20}

Table 1
Elemental analysis, ash content and higher heating value of lignins and lignosulfonates

Samples	N (%)	C (%)	H (%)	S (%)	Ash (%)	HHV (MJ/kg)
Vanisperse	0.12	52.54	4.08	2.96	34.18	20.61
Orzan S	0.09	45.42	5.05	5.15	10.83	18.30
Borremet Ca120	0.14	46.63	5.35	5.62	13.13	19.46
Marasperse N 22	0.14	43.52	4.68	6.28	24.96	18.17
Boresperse N	0.14	44.11	4.65	6.49	23.34	18.36
DP-03	0.14	48.12	5.08	7.15	22.41	17.99
DP-02	0.16	42.86	4.38	5.12	19.64	17.31
Maratan SN	1.27	65.26	7.79	8.73	11.81	19.51
Sulfonated Kraft lignin Westvaco	0.12	44.65	5.99	1.35	23.74	20.67
Lignin Olsany	1.18	63.64	5.93	0.49	0.43	25.88
Kraft lignin Bukoza	0.28	55.68	4.62	3.91	3.85	23.62
Lignin HNO ₃ , pH 3	1.85	62.06	5.91	0.01	1.91	25.23
Lignin HCl, pH 3	1.20	58.69	5.72	0.01	2.13	23.86
Lignin CH ₃ COOH, pH 4.3	1.21	62.48	5.93	0.00	5.99	25.40

To obtain the concentration of individual phenolic structures in mmol/g, the following absorbance-based formulae were developed^{19,20} and applied in their original form in this work:

a) Non-conjugated phenolic structures (I + III)

$$\text{OH(I + III)} = \{ (0.250 \times A_{300 \text{ nm}}(\text{NaOH}) + 0.0595 \times A_{350 \text{ nm}}(\text{NaOH}) \} \times 1/(c \times d) \quad (\text{eq. 1})$$

b) Conjugated phenolic structures (II + IV)

$$\text{OH(II + IV)} = \{ 0.0476 \times A_{350 \text{ nm}}(\text{NaOH}) \} \times 1/(c \times d) \quad (\text{eq. 2})$$

c) Total amount of phenolic hydroxyl groups

$$\text{OH(I + II + III + IV)} = \{ 0.250 \times A_{300 \text{ nm}}(\text{NaOH}) + 0.107 \times A_{350 \text{ nm}}(\text{NaOH}) \} \times 1/(c \times d) \quad (\text{eq. 3})$$

where A_λ – absorbance at a given wavelength divided by the corresponding molar absorptivity, c – mass concentration in g/L; d – pathlength through the sample in cm.

RESULTS AND DISCUSSION

The use of lignin as a fuel in different applications requires knowledge of its heating value. Several studies investigate the heating values of isolated lignins and different biomass species. Pure lignin has a quite higher heating

value than cellulose and hemicellulose, which makes it interesting for using as a bio-fuel. On the average, the higher heating value (HHV) of lignins and lignosulfonates is 21.0 ± 3.1 MJ/kg of dry lignin. The weight percentage of elements C, H, N, S, ash content, and HHV of the isolated

precipitated lignins and commercial lignosulfonates are listed in Table 1.

To investigate the structural components of lignin, UV spectroscopy was used. The ash present in solutions was separated by membrane filtration. To monitor and analyze changes in the conjugated phenolic compounds in isolated precipitated and commercial lignosulfonates, ionization difference UV-Vis spectroscopy was applied. This method is based on the findings that while the UV spectra of etherified lignin units are identical in neutral and alkaline medium, those of

non-ionized (neutral media) and ionized (alkaline media) phenolic hydroxyl groups differ. Subtracting the absorbance curves of such different spectra, the ionization difference spectrum is obtained. Fig. 2 shows that lignins had absorption maxima around 219, 251, 300 and 355 or 365 nm and minima centered at 232, 278 nm and 318 nm. To determine the local maxima and minima, the first derivative of the spectral curves was performed. The obtained values are gathered in Table 2.

Table 2
Local maximum and minimum for 1st derivative of the difference ionization UV-Vis spectra of lignin and lignosulfonates

Samples	Local maximum (nm)				Local minimum (nm)		
Vanisperse	218.5	253	301	357	232.5	281.5	
Orzan S	218	252	301		231.5	279.5	321
Borremet CA120	219	251	300.4	355	231.5	278.5	
Maresperse N 22	219	255	301.5		232	279.7	
Borresperse N	219	251.5	300.5		232.5	280	
DP03	218.5	251.5	300.9	355	231	279	319.5
DP02	219.5	255	293.5		234	279.8	320
Maratan SN	218.5	254.5	295.5	354.5	232.5	279.7	
Sulfonated kraft lignin Westvaco	219	249	303.5	350.5	233	278.5	
Lignin (H ₂ SO ₄ , pH 3)	221	250	300	361	233.5	278	316
Kraft Lignin Bukoza	220	252	296.5	368.5	237.5	277.5	317
Lignin (HNO ₃ , pH 3)	220	248.5	299.7		233	276	317.5
Lignin (CH ₃ COOH, pH 4.3)	219	250.5	300.5	363.5	231.5	277	
Lignin (HCl, pH 3)	219.5	250.5	300.7	354.5	231	277	315

Free and etherified hydroxyl groups contribute to the characteristic absorption minimum of lignin near 280 nm (difference spectrum). The spectral intensity at 280 nm is influenced to a substantial extent by the presence of unsaturated substituents on α -carbon of a side chain (e.g., carbonyl group, double bond). Such unsaturated groups cause absorption in the near UV region (300-400 nm) in the form of broad peaks or shoulders and thus lignin colour.²¹ The absorption minima of unconjugated quaiacyl and 3,4-dimethoxy-phenyl model compounds fall within the narrow wavelength range from 277 to 282 nm. The lignin absorption from 300 to 400 nm is due to carbonyl groups and/or double bonds conjugated with an aromatic ring. Evidence for phenolic conjugated α -keto groups was provided by the difference

method. The ionization difference spectrum for certain lignin samples shows a shoulder or a maximum near 350 nm, which agrees well with the maxima in the spectra of alkaline solutions of model compounds, such as vanillin ($\lambda_{\text{max}} = 353$ nm).²² The intensive band near 340 to 350 nm is consistent with the formation of extensive conjugated structures, such as the $C\alpha=C\beta$ double bond, β -C=O structure, and the conjugated carbonyl group in side chains of lignin, such as coniferyl aldehyde.²³

The peak centered around 355 nm in the ΔA spectrum of preparations is composed of peaks due to p,p-dihydroxystilbenes ($\lambda_{\text{max}} = 356$ nm) and phenolic α -carbonyl units ($\lambda_{\text{max}} > 350$ nm).²² The ratio of absorptivity of 282 nm and 280 nm peaks for purified lignosulfonates and for native

lignins (ratio of percent methoxyl) ranges from 0.92 to 1.1.²⁴ For the isolated lignins, the ratio ranges from 1.01 to 1.12, and for lignin (CH_3COOH , pH 4.3) it reaches the value of 1.61. For the commercial liginosulfonates, a ratio varying from 0.94 to 1.22 is calculated. Lower values of the ratio would indicate the presence of other chromophores, such as conjugated carbonyl²⁵ and diphenyl,²⁶ which absorb more intensely.

Normalized difference UV-Vis spectra of the preparations are shown in Figs. 3 and 4, to facilitate the identification and discussion of similarities and differences among these preparations. These results are plotted for comparison purposes as a ratio of the absorbance in the whole wavelength range versus the absorbance at 220 nm.

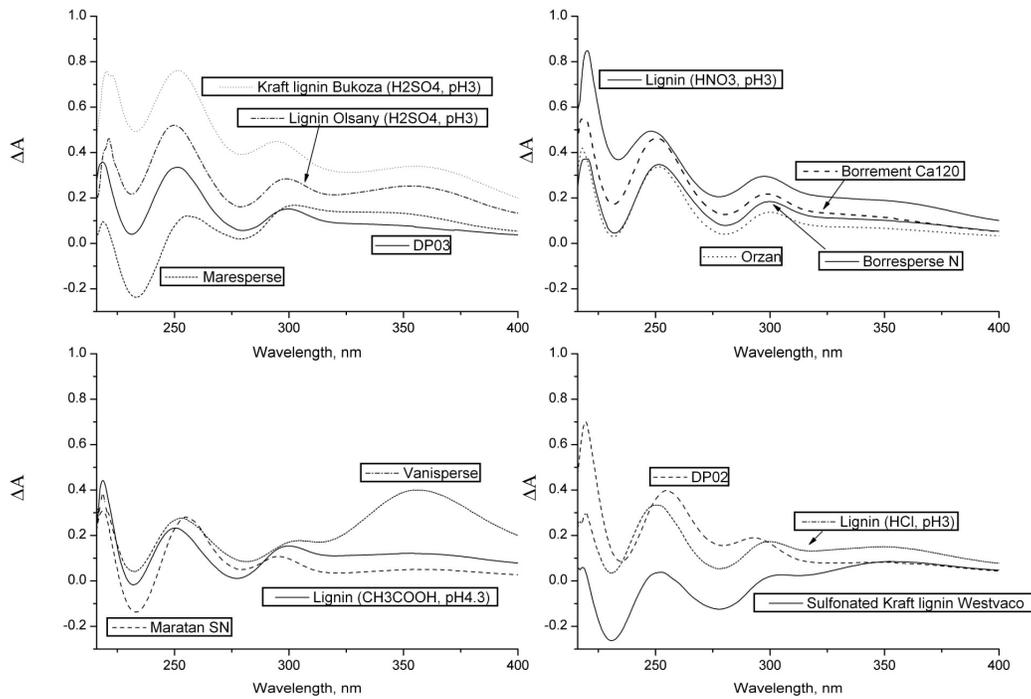


Figure 2: Ionization difference UV-Vis spectra of lignin and sulfonated lignins

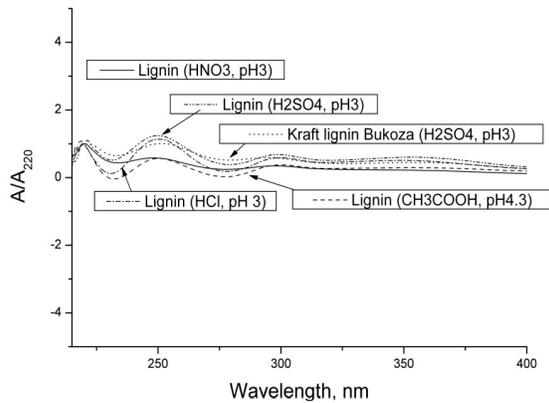


Figure 3: Normalized difference UV-Vis spectra of liginosulfonates

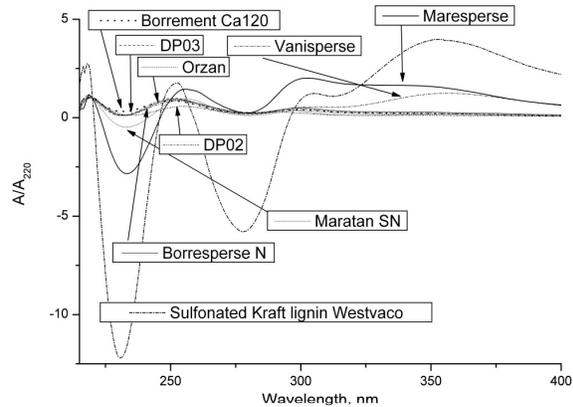


Figure 4: Normalized difference UV-Vis spectra of isolated lignins

In the case of the five commercial lignosulfonates (Borremment Ca 120, DP03, Orzan S, Borresperse N and DP02), the shape of the curve is nearly identical in the whole scanned UV-Vis region. As for Vanisperse, part of its UV-Vis curve is similar to those of the mentioned five samples, there is, however, an increase in the A/A_{220} ratio in the region 300-400 nm. Maratan SN sample exhibits a decrease (-0.52) in the A/A_{220} ratio at 238.8 nm. Even a more profound decrease (-2.85) in the A/A_{220} ratio was observed

for Maresperse at 233.4 nm. On the contrary, at 255.9 nm the A/A_{220} ratio reaches the value of 1.44. When comparing to the previously mentioned samples, the ratio increases in the region 281-400 nm. The most significant changes were found for sulfonated kraft lignin Westvaco. The most considerable decrease (-12.2) among all samples in the A/A_{220} ratio is observed at 230.7 nm, another substantial decrease (-5.77) is at 277.9 nm. In the 297-400 nm region, the ratio increases, reaching its highest value (3.94) at 353.3 nm.

Table 3
Content of non-conjugated and conjugated phenolic hydroxyl groups and their total amount for lignins and lignosulfonates

Samples	Non-conjugated phenolic structures (I+III), mmol/g	Conjugated phenolic structures (II+IV), mmol/g	Total amount of phenolic hydroxyl groups (OH I+II+III+IV), mmol/g
Borremment	1.44	0.13	1.57
Vanisperse	1.49	0.42	1.91
DP2	1.08	0.09	1.18
DP3	1.02	0.09	1.11
Orzan S	0.94	0.08	1.01
Maratan SN	0.62	0.05	0.67
Sulf. kraft lignin Westvaco	0.27	0.11	0.37
Maresperse	1.26	0.16	1.42
Borresperse N	1.36	0.13	1.48
lignin (H ₂ SO ₄ , pH 3)	2.24	0.31	2.55
Kraft lignin Bukoza	3.13	0.39	3.52
Lignin (HNO ₃ , pH 3)	2.20	0.23	2.43
Lignin (CH ₃ COOH, pH 4.3)	1.30	0.16	1.46
Lignin (HCl, pH 3)	1.33	0.18	1.52

The changes in the case of isolated lignins are less distinct than those observed for lignosulfonates, there are, however, observable differences in A/A_{220} . It is obvious that the kind of acid used for precipitation influences the shape of the curves. For the lignins precipitated by HNO₃ or H₂SO₄ no substantial changes in the curve shapes happen. On the other hand, in the lignin samples obtained applying HCl or acetic acid, the changes are more profound. The values for the content of non-conjugated and conjugated phenolic hydroxyl groups, as well as their total amount, in these preparations are presented in Table 3.

In the case of commercial lignosulfonates, the content of non-conjugated phenolic structures (I+III) ranges from 0.27 to 1.49 mmol/g. The highest content of these structures was found in Vanisperse and Borremment, the lowest one in sulfonated kraft lignin Westvaco (0.27 mmol/g).

The highest content (3.13 mmol/g) of non-conjugated phenolic structures (I+III) was determined in kraft lignin Bukoza. The lignin obtained from black liquor from modified alkaline anthraquinone cooking contains 2.24 mmol/g when isolated using H₂SO₄ and 2.20 mmol/g when precipitated with HNO₃. The content of the same groups in the samples isolated by means of HCl and CH₃COOH ranges from 1.30 to 1.33 mmol/g. The content of conjugated phenolic structures (II+IV) varies in the interval of 0.05-0.42 mmol/g with the highest one found in Vanisperse, while for the other commercial lignosulfonates it is 0.11 ± 0.03 mmol/g. The lignin isolated from kraft liquor by H₂SO₄ contains 0.39 mmol/g of conjugated phenolic structures, for the other type of lignin isolated in the same way the content is slightly lower, approaching 0.31 mmol/g.

The total amount of phenolic hydroxyl groups (OH I+II+III+IV) in commercial lignosulfonates is highest in the case of Vanisperse (1.91 mmol/g) and lowest for sulfonated kraft lignin Westvaco (0.37 mmol/g). As for isolated lignins, the highest content of these groups has kraft lignin Bukoza (3.52 mmol/g). Isolation of lignin by different acids led to an evidence that the type of acid influenced both the content of individual groups and the total content of the groups. When using H₂SO₄, the total amount of phenolic hydroxyl groups (OH I+II+III+IV) was 2.55 mmol/g, the use of HNO₃ led to 2.44 mmol/g and isolation by means of CH₃COOH and HCl provided a lower total content, 1.46 and 1.52 mmol/g, respectively. From the results of Table 3, it can be noted that the content of non-conjugated, conjugated phenolic hydroxyl groups and their total amount are different. This can be due to the method, raw material, and experimental conditions used in the pulping process, method of lignin isolation and type of acids. El Mansouri *et al.*²⁷ found that alkaline lignins from rice and wheat straws had higher content of aliphatic and phenolic groups than those of kraft lignin. In this work, it was observed that kraft lignin had a higher content of total amount of phenolic hydroxyl groups than lignin with straw, isolated for modified alkaline anthraquinone cooking with different acids. A different impact of the method of isolation on the content of the mentioned groups was also found in other works.²⁸⁻³⁰ It was proposed that the content of phenolic OH groups, determined by UV difference spectroscopy can be used as an indicator of their potential reactivity.²⁷ The effect of various acids at identical pH on the concentration of individual phenyl hydroxyl groups has been also observed by other authors;³¹⁻³⁷ it has not been, however, unambiguously explained. It is known that when lignin is precipitated from kraft black liquor, the methoxyl content decreases, while the carboxyl and phenolic hydroxyl contents increase at a lower pH.^{36,37} Lowering the pH of the black liquor using different agents (organic or mineral acids, CO₂) protonates the phenolic hydroxyl groups in lignin.^{33,34} The isolation of lignin, the effectiveness of precipitation, and the properties of the obtained lignin (the presence and concentration of individual functional groups) depend on the precipitation conditions (temperature, lignin concentration, ionic strength, etc.), as well as on lignin protonization and the

acid used (e.g., its acidity constant).^{33,35,36} The results obtained contribute to the study on the relationships between the nature of individual phenolic groups, their concentration in lignins and their derivatives, and the antioxidant activity of these compounds. This activity seems to be a new and promising field of application of lignins and their derivatives and deserves due attention.

CONCLUSION

The difference UV-Vis absorption of 5 lignins isolated by precipitation and 9 commercial lignosulfonates was studied. In this work, the difference method has been used for studying the amount of non-conjugated and conjugated phenolic hydroxyl groups and their total amount for lignins and lignosulfonates. It was found that kraft lignin had a higher total amount of phenolic hydroxyl groups than lignin with straw (hemp and flax), isolated from modified alkaline anthraquinone cooking with different acids and commercial lignosulfonates. It was confirmed that the method, raw material, and experimental conditions used in the pulping process, as well as the method of lignin isolation and type of acids, influenced the content of non-conjugated and conjugated phenolic hydroxyl groups, as well as their total amount in the preparations.

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