

Evaluación los distintos procesos oxidativos de la lignina proveniente del pasto venezolano “*Megathyrsus Maximus*”, para activar la energía metabólica.

Evaluation of the different oxidative processes of lignin from the Venezuelan grass “*Megathyrsus Maximus*”, to activate metabolic energy.

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Resumen

Debido al incremento de los costos para la alimentación, actualmente se buscan opciones alimenticias que aporten algunos elementos de interés en la nutrición animal. En esta búsqueda de opciones, se han caracterizado algunas especies de plantas que son palatables (grato al paladar) para los animales y existe poco conocimiento científico sobre su composición química. Por lo anterior, el objetivo de este trabajo es modificar la estructura de la lignina, a través de un tratamiento químico oxidativo, para obtener estructuras de menor masa molecular promedio y se pueda aprovechar como un aditivo en la formulación de alimentación. Utilizando un experimento de diseño cribado 2^3 con dos puntos al centro; temperaturas (30, 65 y 40) °C, concentración de H_2O_2 (5, 7, y 9) % y tiempo (40, 60, y 80) min. Se evaluó los resultados a través de análisis de caracterización de las ligninas oxidadas y sin oxidar, FTIR y UV, obteniendo resultados preliminares de la oxidación de la lignina. Se obtuvo mayor conversión para los parámetros directamente proporcionales a la temperatura y a la concentración teniendo un porcentaje promedio de 16,75% y una conversión menor del 2%, mayor de 73% y se obtuvo un aumento de longitud de onda en 284 nm para la lignina oxidada. Por lo antes mencionado, es necesario generar opciones de alimentación animal de uso eficiente y viable desde el punto de vista ecológico, económico y de fácil adopción, utilizando los recursos disponibles en la región. Por ello es necesaria la modificación de la estructura de lignina, debido que no es digerible por los rumiantes y está incorporada en la dieta alimenticia.

Palabras clave: Lignina, oxidación, rumiante, Energía metabolizable.

Abstract.

Due to the increase in feed costs, alternative feed options are being explored that provide elements of interest in animal nutrition. In this search for options, some species of plants that are palatable to animals have been characterized and little scientific knowledge exists about their chemical composition. Therefore, the objective of this work is to modify the structure of lignin through an oxidative chemical treatment to obtain structures with a lower average molecular mass, which can be used as an additive in feed formulation. Using a 2^3 factorial screening experiment design with two central points: temperatures (30°C, 40°C, and 65°C), H_2O_2 concentration (5%, 7%, and 9%), and time (40, 60, and 80 minutes). The results were evaluated through FTIR and UV characterization analysis of oxidized and unoxidized lignins, obtaining preliminary results of lignin oxidation. Greater conversion was obtained for the parameters directly proportional to temperature and concentration, having an average percentage of 16.75% and a conversion of less than 2%, greater than 73%, and an increase in wavelength of 284 nm was obtained for the oxidized lignin. Due to the aforementioned, it is necessary to generate animal feeding options that are efficient and viable from an ecological, economic and easy adoption point of view, using the resources available in the region. Therefore, modification of the lignin structure is necessary, because it is not digestible by ruminants and is incorporated into the diet.

Keywords: Lignin, oxidation, ruminant, Metabolizable energy.0

1. Introduction.

Lignin is a natural polymer constituent of plants, being the second most abundant polymeric compound in nature after cellulose. It acts as a binding agent for the fibers present in biomass; it is mainly composed of cellulose, hemicellulose and lignin. Due to the characteristics of lignin, which is a renewable, biodegradable and easily available resource, in recent years it has gained momentum in research focused on the exploitation of this resource as a raw material to obtain clean energy and products. value-added chemicals in different areas; pharmacy, food, livestock, oil, among others.

Most of the lignin residues are discarded or burned as low-grade fuel, which is not only a waste of resources but also creates a serious environmental pollution problem. Lignin represents the only renewable source of aromatic chemicals, hydroxyls, phenolics, alcohols, carbonyls, methoxys, carboxyls, and conjugated double bonds (see Figure 1) and the second largest renewable source of carbon. Found in different biomasses as indicated in Table N 1.

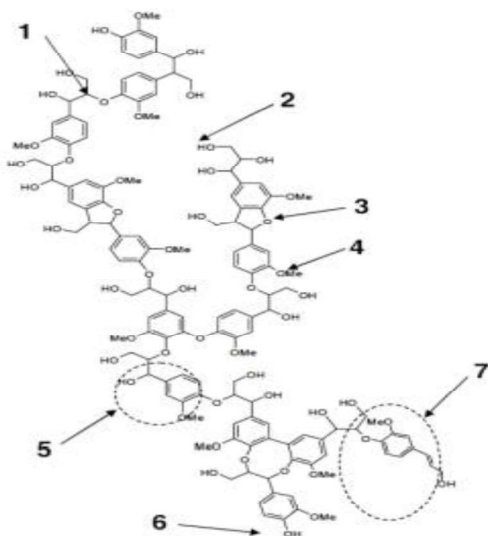


Fig. 1. Reactive points of the lignin molecule. 1.- O-4 bond (aryl ether). 2.- aliphatic hydroxyl. 3.- a-O-4 bond (a-aryl ether bond). 4.- methoxy. 5.- Non-condensed unit. 6.- Phenolic hydroxyl. 7.- Unsaturated unit. (FIRP 2020).

In the structure of lignin (see Figure N 1) the different active points found are indicated, among them the groups: aromatic, hydroxyl, phenolic, alcoholic, carbonyls, methoxys, carboxyls, and conjugated double bonds (Chávez, 2013).

Due to its complex polymeric structure, associated with its isolation, the structure of lignin is not fully defined due to the variation of its origin. However, it is known that it is made up of three basic structural monomers, which are: p-coumaryl alcohol, coniferyl alcohol and sin-

apyl alcohol, corresponding to the phenylpropane units: coupled 1-hydroxyphenyl, guaiacyl and sinapyl.

Table 1. Presence of lignin in different species.

Especie		Contenido de lignina (%)
Nombre científico	Nombre común	
<i>Tsuga canadensis</i>	Abeto Oriental	31,0
<i>Panicum virgatum</i>	Pasto varilla	33,0
<i>Pseudotsuga menziesii</i>	Abeto de Douglas	29,0
<i>Pinus sylvestris</i>	Pino de Escocia	28,0
<i>Pinus radiata</i>	Pino de Monterey	27,0
<i>Prunus amigdalus</i>	Almendro	26,0
<i>Eucalyptus grandis</i>	Eucalipto rosa	25,0
<i>Acacia mollissima</i>	Acacia negra	21,0
<i>Benula verrucosa</i>	Abedul	20,0
<i>Populus tremula</i>	Álamo europeo	19,0
<i>Olea europaea</i>	Olivo	19,0
<i>Gossypium hirsutum</i>	Algodón	16,0
<i>Saccharum species</i>	Bagazo de caña	14,0
<i>Oryza species</i>	Paja de arroz	6,1

(FIRP, 2020).

The bonds between these basic units are attributed to different ether-type bonds, which are: alpha-aryl, beta-aryl, diaryl, phenylcoumarane and alpha-alkyl, as shown in Figure 2. Likewise, there are also bonds of the type bi-phenyl and 1,2-diarylpropane (Garcias, 2022).

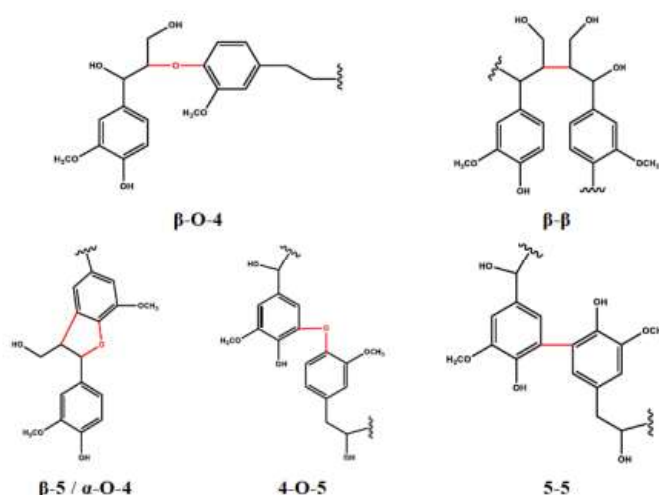


Fig. 2. Major bond types of the units that make up the lignin molecule (FIRP, 2020).

Next, some chromophore groups will be shown that are attributed the coloration due to activation depending on their alkaline extraction (see Figure 3), on the other hand, the coloration is due to the condensation of lignin and the oxidation of phenolic groups.

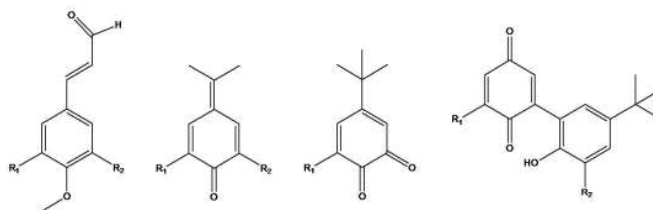


Fig. 3. Chromophore groups that give dark coloration after Lignin extraction (FIRP, 2020).

Due to the aforementioned, different methods have been developed such as: halogenation, nitration, phenolation, graft copolymerization, alkylation, dealkylation, sulfomethylation, acylation, reaction with ammonia, esterification and hydrogenolysis, giving effective results through the active sites of the structure of lignin (Suarez, 2017), (L. Montero, 2015).

Therefore, the direct and efficient conversion of lignin molecules to low molar mass aromatic compounds or monomeric molecules is a great challenge and an interesting opportunity for the future. Due to the aforementioned, the research will be focused on the oxidation of the lignin molecule, and in this way, valorize the lignin waste through a chemical transformation, aligned towards products with high added value that can be incorporated as raw material in food of ruminants, or as a product, is a bet on the future, being a challenge due to the digestibility problem.

From the chemical point of view of digestibility. Biomass is made up of different fibers made of cellulose, hemicellulose and lignin (E. Marquez, 2022). For practical conceptual purposes, it has been defined in terms of Crude Fiber (CF), Neutral Fiber (NDF) and Detergent Acid (FAD), and is used for the prediction of forage quality, dry matter intake, digestibility and energy value of foods. From the point of view of ruminant nutrition, fiber can be defined as the set of plant components that have low digestibility and promote rumination and rumen balance (Calsamiglia, 1997).

Since lignin is a complex macromolecule, with a great variability in the types of bonds and structures (monomeric and oligomeric (mainly dimeric) phenylpropane) that affects the digestibility of ruminants, it is necessary to perform an oxidative chemical reaction of the molecule partially not totally, therefore, it is necessary to use a design of experiment 32 with two points at the center, for the study different variables will be analyzed among them; temperature, time, concentration of the analyte, around 60 and 75% of the bonds between structural units correspond to ether bonds, and within these bonds, the β -O-4 type ether bond, significantly weaker than the rest of the bonds, corresponds to 45 and 62% of all bond modes (Diaz, 2023). From this, we can establish that the lignin macromolecule is susceptible to a wide range of

chemical transformations and its fragmentation reactions can be divided

into hydrolysis, reduction and oxidation (Domingue, 2018).

The feeding of animals (ruminants) is based on a high percentage of biomass consumption (lignin), which are not favorable in degradability, fattening and productivity, so they try to establish maximum limits of fiber, however they are necessary in their diet, since the lack of fiber results in a depression of fat in milk, acidosis, laminitis and displacement of abomasum, due to physical imbalances (lack of ruminal filling) or fermentative imbalances (reduction of ruminal pH), but an excess of fiber reduces feed intake capacity, ration digestibility, ruminal microbial protein synthesis, and energy intake (Hernández, 2023). The quality and content of nutrients present in the feed used for ruminant feeding depends on the rumen degradability, digestibility, concentration of nutrients and the efficiency with which they can be metabolized and utilized by the animal. Physiologically, once the feed is ingested, degradability emphasizes the amount of feed that disappears in the rumen by the action of ruminal microorganisms. Rumen degradation is important in the supply of nutrients in the diet to meet the demands of microorganisms and animal tissues and organs (Vera, 2020).

Due to the aforementioned, the importance of the research is focused on the modification of the lignin molecule by means of an oxidative process, reducing its structure in such a way as to favor digestibility and energy intake.

For the oxidation of lignin, different enzymatic fungal reproduction methods have been used to degrade lignin (Suarez, 2017). However, they behave differentially with respect to the substrate, in addition to the fact that each one has its own physiological requirements, both for its growth and for the production of enzymes, for this particular study it was performed by chemical process. Being one pretreatment of low cost and high lignin removal, with the pretreatment with hydrogen peroxide (H_2O_2), which was implemented in this investigation, which produces the breaking of the lignin walls that make up the outer layer of the lignocellulosic material of the biomass (Suarez, 2017), (Espinoza, 2022), obtaining as a result the best pretreatment conditions for lignin solubilization obtained from the response surfaces, keeping the concentration of the chemical pretreatment at the 3% level and considering a direct interaction between concentration and reaction time. In this context, the general objective of this research was to pretreat lignin by chemical methods, allowing a yield or fractionation of the lignin structure, under an experimental design with two central point

2. Methodology.

The methodology was divided into two stages: oxidation of lignin (L) using H_2O_2 as an oxidizing agent through the experimental design 23 with two central points and a second stage the characterization of lignin L and oxidized lignin (LO), using instrumental methods and physical properties; moisture, ash, solubility, UV and FTIR spectroscopy.

2.1 Oxidative chemical pretreatment with hydrogen peroxide

The lignin pretreatment stage was ground using a tooth type mill, then the ground biomass was sieved with a Sister de Tamizado model equipment with three mesh levels 6, 10 and 30. Using meshes of 3.35, 2 and 0.6 mm. This ensured a homogeneous reaction of the pretreatment with H_2O_2 . A lignin to H_2O_2 ratio of 1:5 was used, and the reaction pH was maintained between 5 and 6 for 4 hours. At the end of the reaction the solid was filtered and dried at 65°C until constant weight.



Fig. 4. Lignin oxidation assembly (LO).

To evaluate the effectiveness of the oxidative pretreatment of lignin, it was performed by means of a two-point design of experiment 23, being the coded values for each level: temperature, H_2O_2 concentration and time, as described in point 2.2 (see Table 2). By placing a lignin/ H_2O_2 ratio in a three-hole balloon heating the system, once the reaction temperature was reached H_2O_2 was added completely in runs (1, 2, 3, 4, 5, 6, 7, 8, 9, 13, 16, and 17) and in runs (10, 11, 12, 14, 15, and 18) H_2O_2 was added in three parts to verify if there is a difference in the addition (see Figure 4).

Once the reaction was completed, the LO precipitate was separated from the remaining precipitate by vacuum filtration. An iodometric titration was performed on the supernatant to quantify the H_2O_2 concentration at the beginning and end of the reaction. The LO (solid) was determined: moisture, ash, solubility, UV and FTIR, for its characterization before and after treatment.

2.2 Experimental design and statistical analysis

The experimental design was a 23 Factorial screening design with two points at the center, the study variables were: temperature, H_2O_2 concentration and time, as shown in Table 2.

Table 2. Range of values studied in the 23 full factorial experimental design for pretreatment with alkaline hydrogen peroxide.

FACTORS	NIVELS		
	-1	0	1
Temperature (°C)	30	45	65
Concentration H_2O_2 (ppm)	5	7	9
Time (min)	40	60	80
Legend	LO-30540	LO-45760	LO-65980

2.3 Solubility of oxidized lignin.

A lignin and LO solubility study was carried out qualitatively with different organic solvents, observing solubility and coloration changes.

2.4 Yield

The yield was calculated with the grams of lignin and LO obtained from the reaction, by UV study using the equations of the straight lines obtained from the curves between the absorbance and mg/L of the different runs.

2.5 Dry matter and ash

Dry matter, lignin and LO, were measured after drying the samples at 105 °C in an oven until constant weight, weighing before and after drying. Ash was determined after burning the dry sample for 5 hours at 500 °C, weighing before and after combustion.

2.6 Measurement of lignin oxidation.

For the determination of LO content in the oxidative pretreatment, the precipitate (solid) was solubilized with 4% NaOH by Ultrasonic Lc-30 H Elma Ultrasonidoy (see Figure 5).



Fig. 5. Ultrasound to solubilize the lignin mixture obtained (LO) with NaOH 4%.

Once the LO was solubilized, the absorbance was measured with a UV Spectrophotometer model Thermo Electron Corporation (Figure 6), at the following wavelengths: 284, 325, and 373 nm, to visualize how the functional groups present varied.



Fig. 6. UV spectrophotometer.

2.6 Obtaining the infrared spectra.

The LO, product of the reaction, was adequately treated to make a structural study by means of Thermo Fourier Transform Infrared Spectroscopy (FTIR).

3. Discussion of Results.

The present study is based on the degradation of lignin by chemical processes using 4% NaOH with phosphoric acid (H_3PO_4). For the oxidative process, H_2O_2 (peroxidation technique) was used at different concentrations, since it was required to partially oxidize the L, applying a design of experiment 23 with two points at the center, the conditions are described in Table 1.

3.1 Oxidative chemical pretreatment with hydrogen peroxide.

The results obtained from the oxidative pretreatment of lignin, for the 18 runs, three variables were evaluated; temperature, H_2O_2 concentration and time, using a design of experiment 23 (Table 4). According to the results of LO for the 18 runs was 16.75%. Obtaining the lowest percentages in runs (2, 4, 7, and 17), using the parameters; LO-65980 (65 °C, 9%, 80 min) and LO-30580 (30 °C, 5%, 80 min), and runs (1, 9, 11, and 18) with the following parameters LO-30980 (30°C, 9%, 80min), LO-

65940 (65°C, 9%, 40min), LO-30980 (30°C, 9%, 80min) and LO-30540 (30°C, 5%, 40min), the highest percent oxidation of LO was obtained.

It can be observed that at concentrations lower than 12.20 mg/L, H_2O_2 resulted in 2 % LO and a low level of oxidation. These results show that the most important study variables are temperature and concentration; however, an adjustment should be made in the preparation of the lignin sample before oxidation, to confirm the most optimal parameters of the variables, through the experimental design.

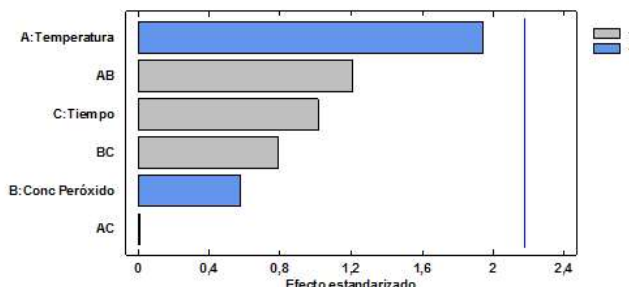


Fig. 7. Statistical Pareto Diagram for Final Lignin Concentration (Author's Source).

The statistical analysis identifies the significant variables and the main effects presented in the experimental design, for the optimization of the process and improvement of the results, it is evident in the pareto that there may be a relevance between temperature and concentration. With a confidence level of 95% using Statgraphic Centurion 16 software (free version), the pareto graph was plotted as shown in Figure 7.

When analyzing Figure 7, the statistically significant variables that exceed the 0.05 magnitude line with a confidence level of 95% are not observed; the variables closest to the magnitude line are temperature and concentration, which are not statistically significant in the work interval studied.

Figure 7 shows that time is not a significant variable; however, the interaction LO-65940 and LO-65540, which corresponds to temperature and H_2O_2 concentration, show the greatest significant effect, as shown in Table 3 and Figure 9.

Table 3. Analysis of variance for conc final lignin.

Source	Sum of Squares	Gl	Medium Square	F-Ratio	P-Value
A: Temperature	11,1222	1	11,1222	3,78	0,0756
B: Conc Peroxide	0,9801	1	0,9801	0,33	0,5744
C: Time	3,0276	1	3,0276	1,03	0,3303
AB	4,30563	1	4,30563	1,46	0,2496
AC	0,000625	1	0,000625	0,00	0,9886
BC	1,8225	1	1,8225	0,62	0,4464
Blocks	5,3976	1	5,3976	1,84	0,2004
Total error	35,29	12	2,94083		
Total (corr.)	61,9463	19			

Table 4. Removal of lignin pretreated with hydrogen peroxide.

Reaction conditions				Pretreatment Result	
Running	Temperature (°C)	Conc. H ₂ O ₂ (mg/L)	Time (min)	Conc. LO (mg/L)	oxidation LO (%)
1	30	5	80	9,89	26,80
2	65	9	40	12,29	2,70
3	30	9	80	6,80	11,70
4	65	5	40	12,46	0,70
5	30	5	40	12,54	7,60
6	65	5	80	10,70	17,20
7	65	7	60	12,45	2,00
8	65	9	80	11,35	10,50
9	30	9	40	7,25	73,00
10	30	5	80	10,92	14,90
11	65	9	40	11,73	30,80
12	30	9	80	11,94	6,40
13	65	5	40	11,08	14,70
14	30	5	40	14,70	4,40
15	65	5	80	12,54	7,60
16	45	7	60	11,76	8,00
17	65	9	80	12,54	2,00
18	30	9	40	10,00	27,10

3.2 Determination of the consumption of oxidized lignin.

Lignin calibration curve, For the determination of lignin concentration (mg/L) of L, the least squares method was used, through the calibration curve of lignin and LO, a correlation index $R^2 = 0.9959$ was obtained, tending to be a perfect straight line, thus fulfilling the Lambert-Beer law,

values of absorbances obtained by UV.

Table 5. Concentration vs. Absorbance for oxidized lignin and unoxidized lignin.

Lignin concentration (mg/L)	Absorbance 373 nm
10,240	0,034
12,800	0,042
15,360	0,052
17,920	0,059

3.3 Spectroscopic studies of modified phenols.

Oxidation of lignin with H_2O_2 is shown. A low cost, high lignin oxidation pretreatment with H_2O_2 , and producing the breakdown of the lignin walls, which form the outer layer of the lignocellulosic material of the biomass, and produces an increase in cellulose accessibility, for subsequent enzymatic attack. Initially the cellulose is covered with hemicellulose and the lignin barrier, which prevents enzymatic attack, the pretreatment with alkaline H_2O_2 , breaks the lignin walls solubilizing it (Suarez J, 2019). Lignin is a very stable polymer so, for its depolymerization it requires treatments under severe conditions of pressure and temperature or reagents (Fraile. Hormigón, 2017), by means of different treatments that will allow us to obtain a large number of compounds, among which are phenols, with respect to the original structure of lignin, results shown in Table 6.

An increase in absorbance, at a wavelength of 284 nm, is observed between lignin and LO. The absorbance is directly proportional to the increase in phenolic concentration in the solution, as a result of oxidative degradation of side bonds and rings (L. Montero 2010), (C. Guillermo, 2020). This may indicate the formation of carboxylic products, phenolic compounds (S. Suarez et al 2019). Caracterización de la lignina oxidada (LO).

Table 6. Absorbance with respect to wavelength (nm) for oxidized lignin and lignin (improve the table).

Sample	284	325	373
Lignin	0,032	0,048	0,034
LO-30580	0,042	0,043	0,030
LO-65940	-	0,060	0,022
LO-30980	0,037	0,038	0,024 ,034
LO-65540	0,037	0,038	0,024 ,034

3.4 Determination of the percentage of ash and moisture in the LO, the determination of the moisture and ash content was carried out on the material on a dry basis, as indicated in the UNE 57050 and ASTM E871-82 methods, Table 7 shows the results obtained for the indicated conditions.

Table 7. Moisture and ash characteristics of the oxidized lignin with H_2O_2 .

Composition	Sample	Humidity ($\pm 1\%$)	Ash ($\pm 1\%$)
Oxidized Lignin (LO)	LO-30980	73,05	7,02
	LO-30940	83,52	17,64
	LO-65580	80,99	10,87
	LO-65540	84,50	10,03

Note: Lignin oxidized for different conditions LO-30980 (T:30 °C, Conc_(H₂O₂): 9%, t: 80 min); LO-30940 (T:30 °C, Conc_(H₂O₂): 9%, t: 40 min); LO-65580 (T:65 °C, Conc_(H₂O₂): 5%, t: 80 min); y LO-65540 (T:65 °C, Conc_(H₂O₂): 5%, t: 40 min).

The results in Table 8 show a high moisture and ash content, only one parameter exceeds the allowed percentage of 10% for biomass in energy production (S. Suarez 2022).

The LO that presented the lowest moisture value of 73.05 % and ash of 7.02 %, was for sample LO-30980, being treated at room temperature 30 °C, higher concentration 9% and longer time 80 min.

3.4 Solubilidad

The 18 LO samples and their solubility results, under different conditions, showed the same behavior as shown in Table 8.

**Fig. 8.** Solubility study for the LO samples.

Table 8. Solubilidad de lignina oxidada (LO) con diferentes solventes.

Muestra	Insoluble	Soluble	Parcialmente Soluble
LO	Agua	NaOH al 4%	alcoholes de cadenas largas
	Octano		
	Tolueno	DMF	Alcohol isopropilico
	Pentanol		
	Butanol		
	Heptano		
	Xileno		

Nota: Lignina oxidadas : LO

tamiento químico que permite disolver en el medio la lignina. Los factores que más influyen sobre este efecto es la masa molecular de la lignina y polaridad del disolvente, por los ligandos en su estructura propuestas siendo macromoléculas (ver Figura 8). En cuanto a los productos de oxidativo de la lignina, se observa diferencias en la disolución al transcurrir el tiempo, modificación en la estructura de la LO observada en el UV y los FTIR.

3.5 Análisis del FTIR.

El espectro FTIR de la Figura 9, muestra las bandas características de la L, observando la zona de huella dactilar de los aromáticos, alcoholes y tensiones C=C, C=O. Para una mejor comprensión se reporta en la Tabla 9 las bandas de absorción, su valor de número de onda y el grupo funcional asociado a la L.

El proceso de oxidación/extracción está relacionado con la solubilidad del compuesto, ya que hay un tra-

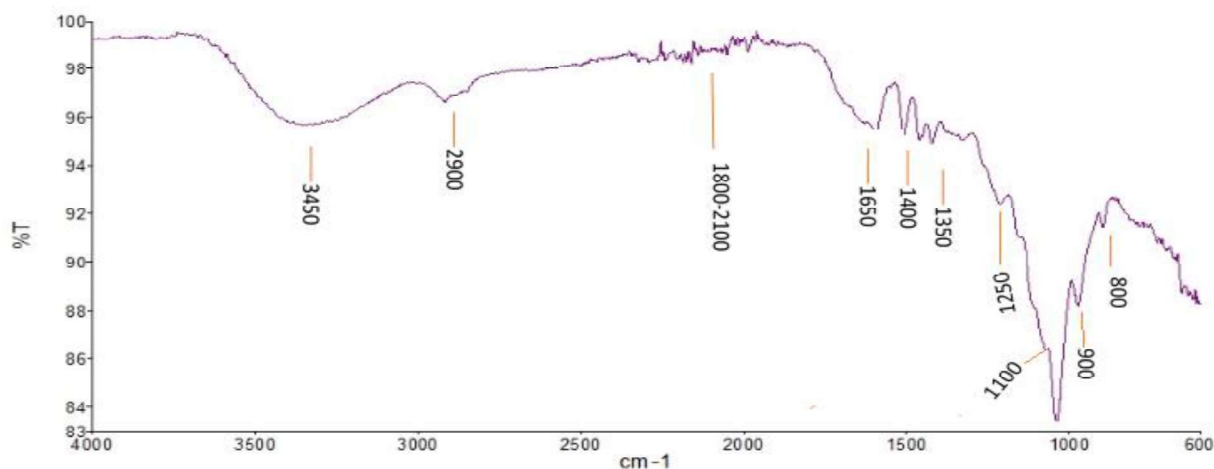
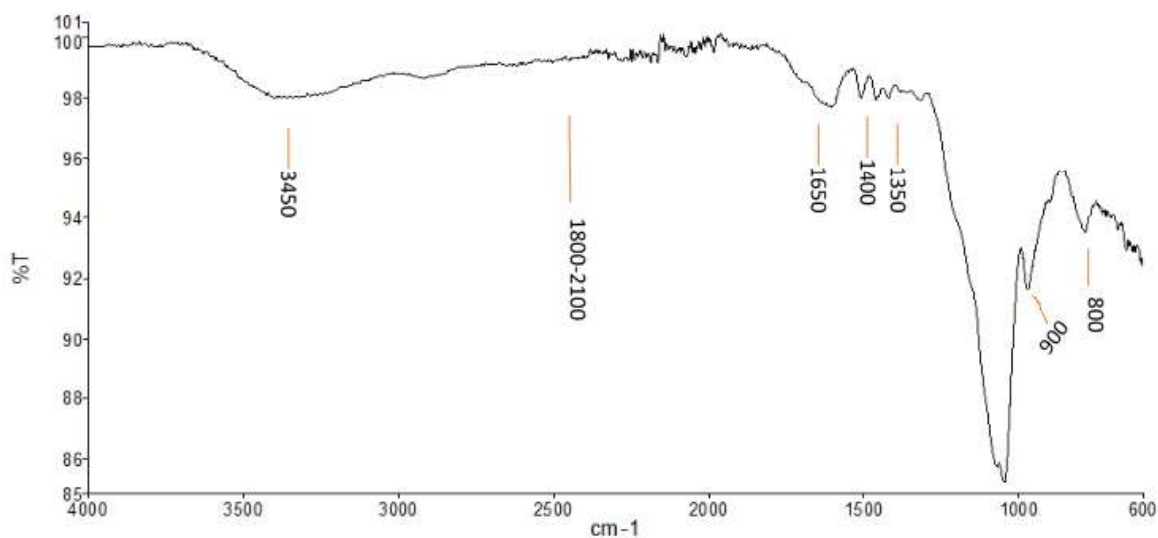
**Fig. 9.** IR spectrum of Lignin extracted with NaOH/H₃PO₄.

Table 9. Infrared absorption bands for lignin.

Sample	ν (cm ⁻¹)	Band	Functional group
Lignin		Voltage -O-H associated	(phenols)
	3450		
	2900	C _{sp3} -H aliphatic	(groups CH y CH ₃)
	2880	CH ₂ symmetrical tension. O-CH ₃ group stretching	(guayacilo- siringil)
	1650-1680	Tension C=O cyclohexanones. Tension O=C-C=C	Aromatic ring bands
	1500-1400	C= aromatic, saturated C-H	Voltage CH ₂ -Ar
	1300	Asymmetric stress Ar-O-C	(guaiacyl-syringyl-4-hydroxyphenyl).
	1250	C-O tension of phenols, vibration of aromatic rings.	Phenols
	1150	Asymmetric C-O stress	Ether
	700-900	C-H aromatic out-of-plane (wide)	H out of plane

Figure 10 shows the FTIR spectrum of the LO, it is evident that there is a modification in the pronounced absorption bands between 1000 to 800 cm⁻¹ with respect to the Lignin of Figure 9, bands corresponding to the car-

bon-oxygen bond (C-O), carbon and hydrogen out of plane (C-H) and a decrease in the intensity of bands is appreciated in 1400 to 1580 cm⁻¹ suitable to C=O tension, O=C-C=C tension and vibration of aromatic rings.

**Fig. 10.** IR spectrum of LO-30980 extracted with NaOH/H₃PO₄

The FTIR spectrum Figure 10, shows a decrease for the LO in the bands between (1100 and 1250) cm^{-1} corresponding to carbon oxygen (C-O) tension of phenols, aromatic ring vibration and in 900 cm^{-1} carbon and hydrogen (C-H) aromatic out-of-plane.

The FTIR spectra (see appendix) obtained for the LO

with H_2O_2 , show the characteristic bands of the L as described in Table 2, the similarities between spectra A-D, and the similarity between spectra B-C, as the existing difference in the spectra is observed in the 800 cm^{-1} wavelength intervals.

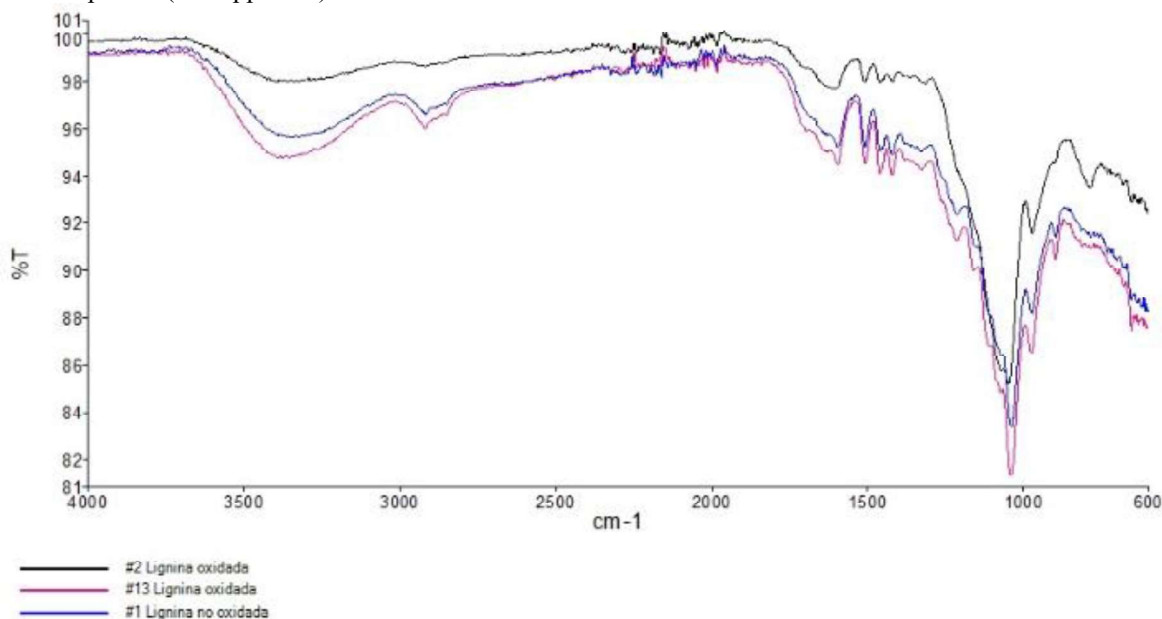


Fig. 11. Espectro ir de lignina oxidada; = lo-65940, = lo-65540 y = lignina.

The FTIR spectrum Figure 11 obtained shows a decrease for sample LO-65940 in the bands between (1100-1250) cm^{-1} corresponding to carbon oxygen (C-O) ten-

sion of phenols, vibration of aromatic rings and in 900 cm^{-1} carbon and hydrogen (C-H) aromatic out-of-plane.

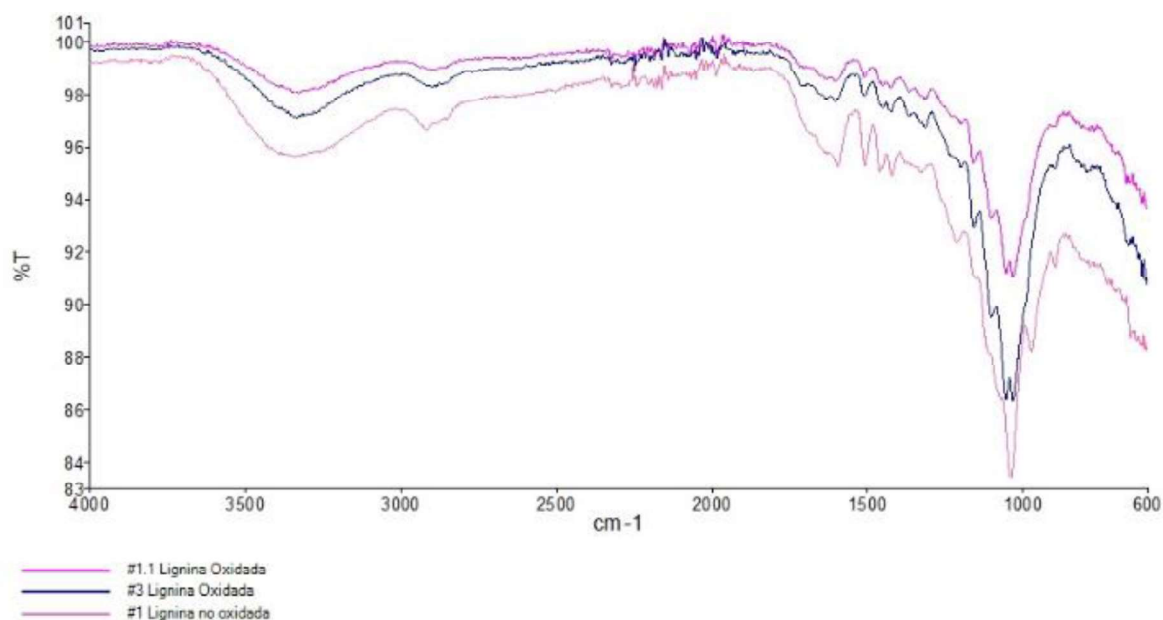


Fig. 12. IR spectrum of oxidized lignin; = LO-30580, = LO-30980 and = unoxidized lignin.

Figure 12 shows the FTIR spectrum of LO; LO-30580, LO-30980 and Lignin, it is evident that there is a modification in the absorption bands pronounced between (800 to 1000) cm^{-1} with respect to lignin, corresponding to carbon-oxygen bonding (C-O), carbon and hydrogen out of plane (C-H) and a decrease in the intensity of bands in (1600-1680) cm^{-1} corresponding to tension C=O, tension O=C-C=C and vibration of aromatic rings.

4. Conclusion.

The pretreatment with H_2O_2 showed a removal of lignin content, and the lignin concentration was quantified in (mg/L). It is important to emphasize that complete removal of lignin is not necessary.

The oxidized lignin macromolecule is solubilized as an effect of the oxidation achieved by means of hydrogen peroxide with respect to temperature and concentration.

The results obtained from the FTIR of lignin oxidation show differences in the wavelengths between (1200-800) cm^{-1} indicating changes in the main bands of the IR fingerprint of lignin.

The variables temperature, concentration, and time were evaluated in a 23 factorial design experiment with two central points at the center, determined the statistical analysis between the significant variables temperature and concentration.

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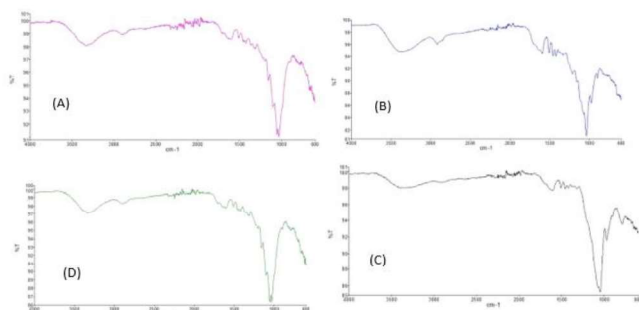


Fig. 13. IR spectrum of oxidized lignin; (a) lo-30580

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