Lignocellulosic characterisation of *Pinus patula* wood from Oaxaca, Mexico

Caracterização lignocelulósica da madeira de *Pinus patula* de Oaxaca, México

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ABSTRACT
Wood is mainly composed of carbohydrates, lignin, and smaller proportions of extractives and minerals. The products that can be obtained and derived from wood depend directly on its chemical composition. This work aimed to determine the contents of α-cellulose, holocellulose, hemicellulose, lignin, and the extractives in ethanol-benzene and ethanol of the wood of six *Pinus patula* trees. An analysis of variance was performed on the obtained data, followed by a multiple comparison of means (LSD, $\alpha = 0.05$). The results indicated significant differences ($p \leq 0.05$) in the chemical compounds among the six trees. On average, *P. patula* wood contained 67.93% holocellulose, 43.28% α-cellulose, 24.65% hemicellulose, 28.08% lignin, 2.30% ethanol-benzene extractives, 0.32% ethanol extractives, and 2.62% total extractives.

Keywords: chemical composition, lignin, cellulose, hemicellulose, extractives.

RESUMO
A madeira é composta principalmente por carboidratos, lignina e pequenas proporções de extrativos e minerais. Os produtos que podem ser obtidos e derivados da madeira dependem diretamente da sua composição química. Este trabalho teve como objetivo determinar os teores de α-celulose, holocelulose, hemicelulose, lignina e dos extrativos em etanol-benzeno e etanol da madeira de seis árvores de *Pinus patula*. Foi realizada análise de variância dos dados obtidos, seguida de comparação múltipla de médias (LSD, $\alpha = 0,05$). Os resultados indicaram diferenças significativas

(p ≤ 0,05) nos compostos químicos entre as seis árvores. Em média, a madeira de P. *patula* continha 67,93% de holocelulose, 43,28% de α-celulose, 24,65% de hemicelulose, 28,08% de lignina, 2,30% de extrativos etanol-benzeno, 0,32% de etanol e 2,62% de extrativos totais.

Palavras-chave: composição química, lignina, celulose, hemicelulose, extratos.

RESUMEN
La madera está compuesta principalmente por carbohidratos, lignina y proporciones menores de extractivos y minerales. Los productos que se pueden obtener y derivar de la madera dependen directamente de su composición química. Este trabajo tuvo como objetivo determinar los contenidos de α-celulosa, holocelulosa, hemicelulosa, lignina y los extractivos en etanol-benceno y etanol de la madera de seis árboles de *Pinus patula*. Se realizó un análisis de varianza sobre los datos obtenidos, seguido de una comparación múltiple de medias (LSD, α = 0.05). Los resultados indicaron diferencias significativas (p ≤ 0.05) en los compuestos químicos entre los seis árboles. En promedio, la madera de *P. patula* presentó 67.93% de holocelulosa, 43.28% de α-celulosa, 24.65% de hemicelulosa, 28.08% de lignina, 2.30% de extractivos de etanol-benceno, 0.32% de etanol y 2.62% de extractivos totales.

Palabras clave: composición química, lignina, celulosa, hemicelulosa, extractivos.

1 INTRODUCTION

Wood from trees is a renewable resource and the primary source of lignocellulosic materials, with a wide variety of applications. It mainly comprises polysaccharides, such as cellulose and hemicellulose, lignin, a heterogeneous aromatic polymer, extractives in minor proportions, and traces of inorganic minerals. In conifers, cellulose constitutes between 40% and 45% of the dry weight of wood, hemicellulose between 20% and 32%, lignin between 25% and 32%, and extractives between 2% and 3.5%. These extractives include a variety of organic compounds such as fats, waxes, pectins, gums, resins, terpenes and essential oils, while inorganic minerals represent less than 1% of the total wood (Rowell *et al*., 2013; Tarasov *et al*., 2018). However, the amount and the type of wood extractives depend on the extraction methods and the solvent used (Trugilho *et al*., 2020; N'Guessan *et al*., 2023).

Bio-based products are estimated to replace 10-15% of petroleum-derived chemicals. Therefore, understanding the chemical composition of wood is essential for producing chemical products, as this composition varies according to the tree's species, age, genotype and growth conditions (Walker, 2006).

Pinus patula Schiede ex Schltdl. et Cham. is native to central Mexico and is found in pine and pine-oak forests in the country's centre and south, at altitudes above 1400 m above sea level. Thanks to its high productive potential and ability to adapt to various climatic conditions and non-forest soils, it is widely used to produce sawn wood. In addition, it has a high value in the paper

industry due to the length of its fibres. For these reasons, it is grown in commercial plantations in South Africa and several South American countries (CONAFOR, 2022).

This study aimed to analyse the chemical composition of the wood of six *Pinus patula* trees, determining the content of its main components: α-cellulose, holocellulose, hemicellulose, and lignin, as well as extractives in ethanol-benzene and ethanol. This characterisation will allow a better understanding of the properties of this species' wood for its use in the production of derived products, such as paper and bioproducts.

2 MATERIALS AND METHODS

Sample processing

The wood of six *Pinus patula* trees from Ixtlán de Juárez (17º 25' 00.04" N, 19º 26' 52.13" W), Oaxaca, Mexico, was analysed. The trees were felled and sectioned into logs 2.50 m long. From the first log of each tree, specifically from its central part, pieces of wood with a square section of 7 cm on each side were obtained, and one piece was randomly selected. The chosen piece was divided into sections 30 cm long, from which one section was also selected randomly. Each section was then planed on a Mizutti electric bench wood planer with two knives to obtain shavings and then left to dry at room temperature. Afterwards, the wood shavings were ground in a Thomas Wiley-type mill and sieved using No. 40 (0.42 mm) and No. 60 (0.25 mm) meshes. The ground wood retained on the 60 mesh was used to determine extractives, lignin, holocellulose, and cellulose.

Extractives determination

The extractive content was carried out sequentially, according to TAPPI standard 204 (TAPPI, 2007). 4 g of oven-dry ground wood was weighed in an extraction thimble, which was placed in a Soxhlet system at reflux for 8 hours with 250 ml of a fresh ethanol-benzene mixture (1:2 v/v). Subsequently, the sample was washed with ethanol under vacuum to remove benzene residues, and a second extraction was carried out using 250 ml of 96% ethanol for 6 hours. Then, it was washed with water to remove ethanol residues, and finally, a third extraction was carried out using 250 ml of distilled water for 6 hours. The samples were dried in an oven at 105 °C before being weighed. The amount of extractives was calculated with the following expression (1):

$$Extracts\ (\%) = \frac{W_e - W_b}{W_s}\ x\ 100 \qquad (1)$$

where
We = oven-dry weight of extract (g)
Wb = oven-dry weight of blank residue (g), and
Ws = oven-dry weight of sample (g)

Determination of lignin

The lignin content was determined following the procedure of TAPPI standard 222 (TAPPI, 2007). 1 g of ground sample free of extracts was weighed, to which 15 ml of 72% sulfuric acid (24 N) was added. The mixture was kept under constant stirring in a water bath at 20°C for two hours. Then, the mixture was transferred to an Erlenmeyer flask with 575 ml of distilled water. The flask was connected to a condenser and boiled for 4 hours on an electric plate inside a fume hood. The solid was then allowed to settle in an inclined position for 24 hours before filtration. The retained lignin was transferred to a filtration crucible, washed with 100 ml of hot distilled water to remove acid residues and dried in an oven at 103 °C for 24 hours before being weighed. The lignin content was calculated using the following expression (2):

$$Lignina\ (\%) = \frac{W_L}{W_s}\ x\ 100 \qquad (2)$$

where
W_L = oven-dry weight of lignin (g), and
W_s = oven-dry weight of the sample (g)

Determination of holocellulose

Holocellulose was determined using the acid chlorite method (Rowell *et al*., 2013). 2.5 g of extractive-free wood was weighed into 250 ml Erlenmeyer flasks, and 80 ml of hot distilled water, 0.5 ml of acetic acid and 1 g of sodium chlorite were added. The flasks were placed in a water bath at 75 ± 5 °C for 1 hour. This procedure was repeated three more times, adding 0.5 ml of acetic acid and 1 g of sodium chlorite on each occasion until 4 hours had elapsed. At the end, the samples were cooled in cold water (<10 °C), filtered and washed with distilled water until the yellow colour and chlorine dioxide odour were removed. Finally, they were washed with 150 ml of ethanol and acetone and dried in an oven at 40 °C. The holocellulose content was calculated with the following expression (3):

$$Holocellulose\ (\%) = \frac{W_r}{W_s} x\ 100 \qquad (3)$$

where
Ws = oven-dry weight of residue, g, and
Ws = oven-dry weight of sample, g.

Determination of alpha-cellulose

To determine α-cellulose, ASTM D1103 was followed. 2 g of anhydrous holocellulose was weighed and placed in a 250 ml beaker. 10 ml of a 17.5% NaOH solution was added, and the holocellulose was macerated with a glass rod until the particles separated. Then, at 5-minute intervals, 5 ml of 17.5% NaOH was added, making a total of 25 ml. The mixture was allowed to stand in a water bath at 20 °C for 30 minutes, then 33 ml of distilled water was added and allowed to stand for one hour before filtering. For washing, the holocellulose residue was transferred to a pre-weighed filtration crucible, washed with 100 ml of 8.3% NaOH, rinsed with distilled water and treated with 15 ml of 10% acetic acid for 3 minutes. It was then rewashed with 250 ml of distilled water to remove traces of acetic acid and dried in an oven at 105 °C. The α-cellulose content was calculated as follows:

$$\alpha - Cellulose\ (\%) = \frac{W_2}{W_1}\ x\ 100 \qquad (4)$$

where
W_2 = oven-dry weight of α-cellulose residue (g), and
W_1 = oven-dry weight of the holocellulose sample (g)

Determination of hemicellulose

Hemicellulose content was calculated as the difference between the holocellulose and α-cellulose contents.

Data analysis

The statistical analysis of the data was carried out using the computer software SAS (SAS, 2000) in conjunction with an analysis of variance (ANOVA) and followed by a multiple comparison test of means using the least significant difference (LSD) method (α = 0.05) (Montgomery, 2017)

3 RESULTS AND DISCUSSIONS

The chemical composition of wood within species varies depending on tree age, selection, geographic region, climatic and soil growth conditions, part of the tree, and silvicultural practices, among other factors (Berrocal *et al.*, 2004; Waliszewska *et al.*, 2015; Darmawan *et al.*, 2018). In this study, there were also significant differences ($p \leq 0.05$) in the contents of the principal and extractive components of the wood among the six *Pinus patula* trees (Table 1).

Table 1. Chemical components of *Pinus patula*

Tree	Lignin (%)	Holocellulose(%)	Cellulose (%)	Hemicellulose (%)
T1	31.89 (1.32) a†	66.23 (0.60) d	41.34 (0.38) d	24.89 (1.20) b
T2	27.75 (0.72) c	71.08 (0.81) a	45.18 (0.59) a	25.89 (1.29) a
T3	28.24 (0.30) b	65.43 (0.92) e	41.68 (0.49) d	23.75 (1.81) c
T4	25.61 (0.59) e	69.44 (0.61) b	44.42 (0.78) b	25.01 (0.46) b
T5	27.20 (0.32) d	67.49 (1.01) c	43.70 (0.66) c	23.78 (1.98) c
T6	27.78 (0.50) c	67.90 (0.71) c	43.32 (0.95) c	24.58 (1.33) b
General Average	28.08 (6.94)	67.93 (2.93)	43.28 (3.31)	24.65 (3.32)

Source: Prepared by the authors (2024).
Values are moisture and extractive-free wood. (Coefficient of variation in parenthesis)
†Values with the same letter are not significantly different ($p \leq 0.05$)

Regarding the lignin content, tree T1 presented the highest percentage, with 31.89%, followed by tree T3, with 28.24% and trees T2 and T6, with values of 27.75 % and 27.78 %, respectively. The lowest lignin content was observed in tree T4, with 25.61%.

Holocellulose content was highest in tree T2, with 71.08%, followed by T4, with 69.44%, while tree T3 presented the lowest value, with 65.43%. For α-cellulose, tree T2 again had the highest content, with a value of 45.18%, while trees T1 and T3 showed the lowest values, with 41.34% and 41.68%, respectively. As for hemicellulose, tree T2 stood out with the highest content (25.89%), followed by trees T4, T1 and T6, with values of 25.01%, 24.89% and 24.58%, respectively, while in trees T5 and T3 the respective values of 23.78% and 23.75 were the lowest.

The lignin values in this study ranged between 25% and 31.89%, which are within the range reported by Bernabé *et al*. (2012) for *Pinus leiophylla*, *P. michoacana* and *P. montezumae*, with a variation between 24% and 28.5%. Similarly, Fonseca (2006) reported values of 22% to 34% for *P. maximinoi*, and Lima (2013) found values between 28.07% and 29.94% for *P. montezumae*, *P. pseudostrobus*, *P. leiophylla* and *Abies religiosa*. In this study, holocellulose values were in the range of 65.43% to 71.07%; these values are lower than those reported by Lima (2013) (79.06% to 81.43%) but similar to those of Bernabé *et al*. (2012) (68.1% to 74.7%) for three pine species. Cellulose contents varied between 41.3% and 45.18%, which are higher than those reported by Revilla (2011) for *P. cembroides*, *P. johanns*, *P. maximartinezzi* and *P. pinceana* (35.5% to 37.9%), although lower than those of Bernabé *et al*. (2013), who reported between 46% and 59%. The obtained values of hemicellulose ranged between 23.75% and 25.89%. These values are lower than those Revilla (2011) reported for four pine species, which varied between 29% and 32%.

Regarding the ethanol-benzene extractives, tree T1 had the highest content, with 3.28%, while trees T6 and T3 had the lowest values, with 1.79% and 1.62%, respectively (Table 2). Concerning the ethanol extractives, tree T4 showed the highest content, with 0.40% and tree T1 had the lowest, with 0.21%. As regards the total extractives, trees T1 and T4 had the highest values, with 3.5% and 3.24%, respectively, while tree T3 showed the lowest content, with 1.93%.

Table 2. Extractives of *Pinus patula*.

Tree	Ethanol-benzene	Ethanol	Total
T1	3.29 (3.45) a†	0.21 (11.18) c	3.50 (3.58) a
T2	2.04 (1.59) c	0.34 (3.50) a b	2.38 (1.67) b
T3	1.62 (4.82) e	0.32 (14.86) b	1.94 (4.93) d
T4	3.03 (1.92) b	0.40 (12.95) a	3.42 (2.07) a
T5	2.01 (2.17) c	0.30 (30.33) b	2.32 (3.42) b
T6	1.80 (3.59) d	0.36 (15.17) a b	2.16 (4.98) c
General Average	2.30 (27.90)	0.32 (23.52)	2.62 (23.97)

Source: Prepared by the authors (2024).
Values are moisture-free wood. (Coefficient of variation in parenthesis)
†Values with the same letter are not significantly different ($p \leq 0.05$)

The content of extracts in ethanol-benzene in this study was from 1.62% to 3.29%; in ethanol, it was from 0.21% to 0.40% and in total extracts, they were from 1.94% to 3.50%; these values are lower than those reported by Lima (2013) and Bernabé (2013) for pine species of 7.9% to 9.1%, 7.6 to 8.2 % respectively.

The variability in the chemical composition of the wood among the different *Pinus patula* trees analysed reflects the inherent heterogeneity of this species. However, some specimens are distinguished for their high levels of certain compounds. For example, tree T1 has a high lignin content (31.89%), while tree T2 stands out for its higher concentrations of holocellulose (71.07%), α-cellulose (45.18%) and hemicellulose (25.89%). Likewise, tree T4 exhibits the highest content of ethanol-benzene extractives (3.28%). These results suggest significant potential for industrial applications in products derived from lignin, cellulose and hemicellulose, as well as higher natural resistance to biological degradation.

4 CONCLUSIONS

Pinus patula wood showed significant differences in its chemical composition among the analysed trees. The lignin content ranged from 25.61% to 31.89%, while holocellulose varied between 65.43% and 71.08%, α-cellulose between 41.34% and 45.18%, and hemicellulose between 23.75% and 25.89%. The total extractives content ranged from 1.94% to 3.50%.

These results suggest that *Pinus patula* wood possesses properties suitable for various industrial processes, including paper manufacturing, biofuel and chemical compound production, as well as the extraction of products derived from lignin, α-cellulose, hemicellulose, and extractives.

It's important to note that these results are specific to the geographic area where *Pinus patula* grows, and the chemical composition of its wood may differ in other regions.

To gain a comprehensive understanding of the wood characteristics of this species, it is recommended to expand the wood characterization to trees from different regions and conduct comparative studies to identify potential variations in chemical composition. Additionally,

analysing the compounds of the extractives and their respective percentages in the wood of this species is also suggested.

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