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ORIGINAL PAPER

Delignification of Toquilla Straw Pulp (*Carludovica palmata*) with a Ligninolytic Enzyme Extract obtained from *Pleurotus ostreatus* and ABTS as a mediator

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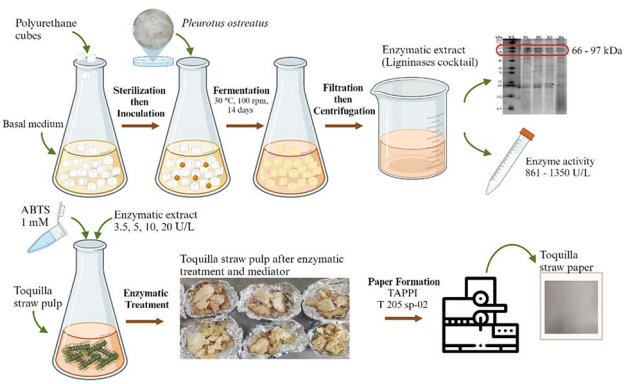
Abstract

Ligninolytic enzymes from basidiomycete fungi are of special interest in the pulp and paper industry for delignification processes, since they promote depolymerization of lignin in cellulosic pulps by oxidation. Among these enzymes are laccases, which oxidative power is favored by chemical or natural mediators. In this research, a ligninolytic enzyme extract, obtained from *Pleurotus ostreatus* was applied along with ABTS as a chemical mediator, to delignify toquilla straw (*Carludovica palmata*) pulp. The enzyme extract was obtained from fermentation of a nutrient medium in polyurethane support. Enzymatic activity was analyzed in the extract, while laccases were assessed through electrophoresis. Extract dosages were of 3.5, 5, 10 and 20 U/g pulp along with 1 mM ABTS. Delignification conditions were 45 °C, pH 4.5 and 200 rpm for 120 h. Ligninolytic enzyme activity was between 861.57 and 1350.69 U/L, and molecular sizes ranged from 66 to 97 kDa. According to a surface response analysis, 14 U/g pulp of enzyme extract and 1 mM ABTS reached maximum delignification, with a final lignin content of 5.04%. The enzyme – mediator system (EMS) caused negative effects on pulp coloration, which was rectified through a subsequent bleaching phase. Finally, the explosion resistance was 15% higher in paper made from EMS pulp, in comparison to the control treatment. Other properties such as the resistance to air flow and width did not present significant differences. The application of enzymatic extracts and a chemical mediator turned out to be an alternative to delignify toquilla straw pulp.

Graphical Abstract



Highlights

- Delignification of Toquilla pulp by enzymes extracts is an environmentally friendly.
- Paper enzymatically treated has less lignin than paper chemically treated.
- The enzyme-mediator system produces a pulp that requires a subsequent washing.

Extended author information available on the last page of the article

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Keywords Delignification · Toquilla straw (*Carludovica palmata*) · *Pleurotus ostreatus* · Ligninolytic enzymes · Chemical mediator

Statement of Novelty

Toquilla straw coming from mature leaf pods that is not used in the artisanal fabrication of hats, but becomes a waste, is evaluated as a no timber source of cellulose pulp. For delignification of this pulp it is been studied an enzymatic treatment by using a ligninolytic extract obtained from *P. ostreatus* and ABTS as a chemical mediator.

Introduction

Global production of paper and cardboard reached 414 million tons in 2022, with an increased demand of 4% compared to the previous year [1, 2]. Wood species are the main raw material for paper making; however, its overexploitation has jeopardized forest resources and caused environmental damage [3]. Non-wood vegetable fibers are an alternative source of cellulosic material for the pulp and paper industry. Vegetable fibers have advantages over wood species, such as lower lignin contents, shorter planting periods, and in general, less consumption of water, energy, and chemical substances including pulping and bleaching agents [4].

Toquilla (*Carludovica palmata*) straw is a fibrous non-woody species, cultivated in moist tropical forests with high sunlight [5]. Fibers, extracted from the plant, are long, smooth, with high tenacity, and present a diameter that ranges from 10 to 20 µm [6]. Toquilla fibers from immature leaf pods or buds are widely used in the Coast region of Ecuador (Jipijapa—Manabí) by country families for the artisanal fabrication of hats; while fibers that come from mature leaf pods are not currently used and could be a renewable source for cellulose pulp [7].

In the paper industry, cellulose pulp is obtained from wood or vegetable fibers through a process known as "pulping", which consists of the removal of lignin from cellulose and hemicellulose polymers [8]. Lignin is an irregular and insoluble polyphenolic polymer that gives stiffness to the cell wall. Its chemical composition of *p*-hydroxyphenyl, guaiacyl, and syringyl units confers lignin a recalcitrant or difficult degradation nature [9]. Therefore, pulping is carried out through physical methods using mills and agitators, or chemical methods with acidic or alkaline solutions. For instance, kraft pulping consists of placing the lignocellulosic fibers in solutions of sodium sulfide (Na_2S) and sodium hydroxide (NaOH), under certain pressure and temperature conditions [10]. In general, long, and strong cellulose fibers are obtained through an appropriate combination of physical and chemical processes, which will then provide resistance properties to the end-products [11].

During pulping, lignin is not eliminated completely, thus traditional chemical methods include subsequent delignification and bleaching operations involving chlorine (Cl_2), chlorine dioxide (ClO_2), hydrogen peroxide (H_2O_2), and ozone (O_3). These delignifying agents remove the residual lignin from the pulp [10]. However, effluents produced after traditional bleaching contain halogenated organic compounds (AOX) like dioxins and furans, which are highly toxic, recalcitrant, mutagenic and bioaccumulate in biological systems [12]. Currently, global research has focused on novel techniques that reduce discharges of AOX in industrial effluents, decrease water consumption, and improve efficiency in pulp production [13].

One of the current novel techniques includes the use of fungi, specifically white and brown rot basidiomycete species, due to their ability to secrete extracellular ligninolytic enzymes [14]. These enzymes are of special interest in the pulp and paper industry, mainly in bio-pulping and bio-bleaching processes, because they oxidize and remove lignin phenolic and non-phenolic compounds [8, 15]. According to their oxido-reductive (redox) action characteristics, ligninolytic enzymes are classified as: laccases (LAC), lignin peroxidases (LiP) and manganese peroxidases (MnP) [16]. Laccases are multiple copper polyphenoloxidases that use molecular oxygen to oxidize phenolic compounds, while LiPs and MnPs act on phenolic and non-phenolic compounds of lignin, but their production is limited to the availability of hydrogen peroxide, manganese, nitrogen, and an appropriate chelating agent [17–19]. Lignin degradation through enzymatic treatment is an environmentally friendly alternative, that reduces the emission of toxic effluents, as well as the energy and chemical input requirements [11].

Previous studies on the use of LACs have found that, in the absence of the living organism, the enzyme extract does not meet the characteristics of a complete biological system for the depolymerization of lignin. For this reason, low molecular weight substances are used to increase the redox action of LACs. These substances are called chemical mediators and may be of synthetic origin such as 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), or 1-hydroxybenzotriazole (HBT), and of natural origin such as syringate, vanillate or syringaldehyde [20]. ABTS is one of

the most studied mediators, which in the presence of LAC acts as an electron shuttle and is capable of degrading phenolic and non-phenolic compounds of lignin, in addition to preventing its repolymerization [21]. The enzyme-mediator system (EMS) results in a reduction of the lignin content in short times, compared to the direct application of the microorganism or enzymatic extract on the fiber [11].

Pleurotus ostreatus is a basidiomycete characterized by its ability to grow, colonize, and synthesize ligninolytic enzymes in fermentative substrates [15, 16]. Some studies showed that using enzymes produced by basidiomycete fungi such as *Pleurotus eryngii* and *Trametes versicolor*, in concentrations of 30 U/g lignin and 10 U/g pulp, respectively, and in the presence of chemical mediators, lignocellulosic pulp delignification was achieved [11, 22]. Another bleaching initiative, focusing on optimizing the laccase-mediator system, found that using laccase in 20.3 U/g of bagasse pulp, 1.51% HBT as a chemical mediator and 154.5 min reaction time, resulted in paper of 17.84% increased brightness, 36% reduced kappa number, ad 23% reduced AOX compared to the control without enzymatic treatment [13].

The aim of this work is to evaluate the enzymatic delignification of cellulose pulp from toquilla straw using a ligninolytic extract obtained from *P. ostreatus* and ABTS as a chemical mediator at laboratory scale. This seeks to determine the dosage of enzyme – mediator that maximizes lignin removal from toquilla straw pulp, and next, apply this dosage to the pulp to prepare paper sheets and analyze their physical properties.

Materials and Methods

Microbial Culture and Ligninolytic Enzymes Production

A basidiomycete fungus *Pleurotus ostreatus*, strain 202, was procured from the culture collection of the Bioprocesses Laboratory, at Escuela Politécnica Nacional (Quito, Ecuador). Fungal cultures were maintained on malt extract agar (MEA) plates at 30 °C and stored at 4 °C [23]. Ligninolytic enzymes production was carried out in basal liquid medium using polyurethane cubes ($1 \times 1 \times 1$ cm^3) as inert support. The basal medium contained glucose (10 g/L), yeast extract (5 g/L), KH_2PO_4 (0.6 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), K_2HPO_4 (0.4 g/L), $CuSO_4 \cdot 5H_2O$ (0.25 g/L), $FeSO_4 \cdot 7H_2O$ (0.05 g/L), $MnSO_4 \cdot H_2O$ (0.001 g/L) and $ZnSO_4 \cdot 7H_2O$ (0.001 g/L) as described by Téllez-Téllez et al. [24]. Five disks (5 mm in diameter) from the growing edge of the mycelium on MEA plates were transferred to 250-mL Erlenmeyer flasks, each containing 35 mL of the basal liquid medium and 1 g of the inert support cubes. In total, 3 batches of 22 flasks were incubated at 30 °C in a shaking bath at 100 rpm. Culture supernatants were harvested on day 14 and filtered through cheesecloth [25]. The filtrate was then centrifuged at 3000 rpm for 20 min and used as a source of ligninolytic enzymes. Cell-free supernatants were stored at -14 °C until they were used.

Enzyme Assays

Laccase activity was determined through oxidation of ABTS method. Increase in absorbance was measured spectrophotometrically for 3 min at 420 nm (ε_{420} 3.6×10^4 $M^{-1}cm^{-1}$) and 30 °C. The reaction mixture contained 100 µL of 50 mM ABTS (Sigma-Aldrich), 800 µL of 20 mM sodium acetate buffer (pH 4.5), and 100 µL of diluted enzyme extract. Enzyme activity was expressed in units (U); one unit is equivalent to one µmole of ABTS oxidized per min [26].

Enzyme Molecular Characterization

SDS-PAGE vertical electrophoresis was performed to determine enzymatic purity and approximate molecular mass of laccases. Lyophilized enzyme extracts were characterized through electrophoresis according to Schägger & von Jagow [27] methodology. Broad range molecular weight markers (SIGMA-S8445-10VL) were used as standards.

Raw Material and Pulping

Toquilla stalks were obtained from a local farm (Puerto Quito, Ecuador). Fiber was separated from stalks through a mechanical shredder and cooked for 15 min at 100 °C. The cooked fiber was then drained and oven dried for 48 h at 40 °C. Fiber was characterized for moisture, lignin and cellulose content, following standard methods ASTM D1106-96, TAPPI T 17 wd-70, and TAPPI T 550 om-08 [28–30]

The basic pulp extraction method was as follows: 750 g of toquilla fiber were cooked in a digester (M/K D2015-E2-580, USA) with 4% sodium hydroxide solution (white liquor), at a ratio of 1:8 (w/w), fiber to liquor [31]. The mixture was heated to 160 °C and the reaction takes place for 12 min. The pulp obtained from the digestion was washed at 90 °C for 60 min, then dried at 60 °C for 24 h, and weighed.

Pulp Treatment with Enzyme-Mediator System (EMS)

Pulp treatments with laccase and ABTS as mediator (EMS) were carried out in 250-mL Erlenmeyer flasks with 3.5 g (dry weight) of pulp at 2% consistency in 50 mM sodium tartrate solution, pH 4.5 [23]. Four enzyme concentrations: 3.5; 5; 10 and 20 U/g of pulp were tested, along with 1 mM of ABTS [32, 33]. Control treatments were performed with pulps treated under the same conditions but without enzyme,

mediator, or both. The reaction mixture flasks were incubated at 45 °C and 200 rpm for 120 h [33].

After the reaction, pulps were washed with distilled water and further bleached with 50 mM NaOH and 2.5% (w/v) H_2O_2 at 60 °C for 1.5 h with shaking. Treated pulps were filtered and oven dried at 60 °C for 24 h and packed in air-tight bags until they were analyzed [22, 33, 34].

Analytical Studies

The effect of EMS on delignification of pulp was assessed by estimating the kappa number according to TAPPI T 236 [35], as well as color properties through CIE L*a*b coordinates [36]. All determinations were done in duplicates and the reported results are an average of the two runs. Results were analyzed through a factorial ANOVA design using Statgraphics Centurion XVIII. Differences among treatments were determined according to the LSD test. P values less than 0.05 were statistically significant. Optimum dosage of enzyme and ABTS that maximizes lignin removal was determined by the response-surface method [37].

Pulp Characterization

Pulp was treated under the optimized conditions for enzyme and ABTS concentrations, derived from the response-surface analysis. Kappa number (residual lignin), cellulose content were studied in pulps after the treatment, according to standard protocols: TAPPI T 236 and TAPPI T 17 wd-70 [29, 35]. Also, the color properties were analyzed [36].

Paper Sheet Formation

Following the optimized enzymatic-mediator treatment, pulp was formed into paper sheets of approximate 28 cm × 28 cm, according to standard methods TAPPI T 205 sp-02 [38]. ASTM standards were used to evaluate properties such as thickness [39], mass per unit area [40], resistance of paper to passage of air [41] and bursting strength [42].

Results and Discussion

Enzyme Extract

Each batch produced around 600 mL of crude enzyme extract, showing an average laccase activity (AE) of 1157.79 U/L. In the SDS-PAGE electrophoresis analysis, molecular weights from four samples of enzymes extracts (M1—M4) from different fermentation batches are displayed in Fig. 1. Bands were observed throughout the entire length of lanes and compared against a broad-range (WR) pattern. The four samples indicate the presence of

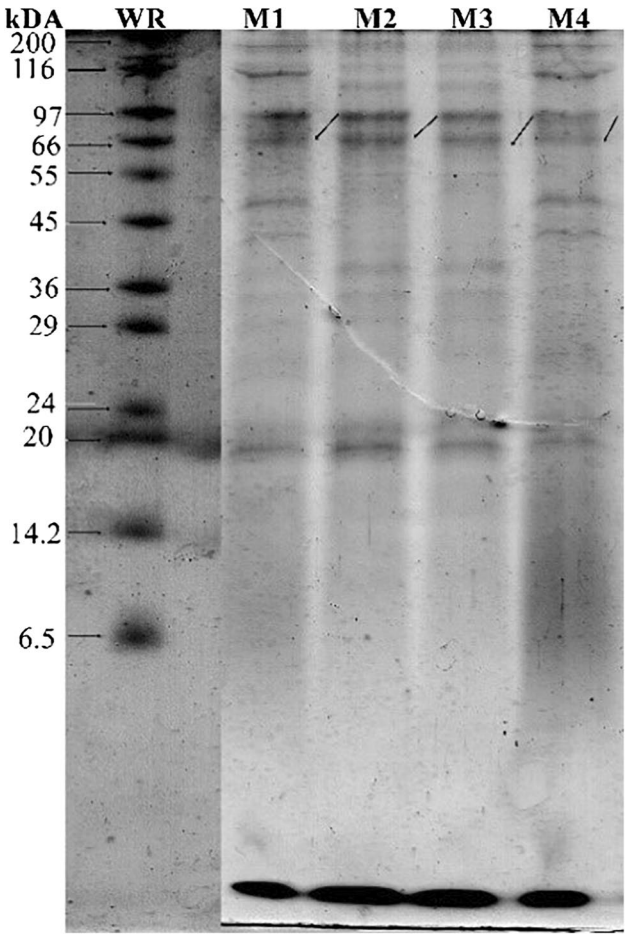

Fig. 1 Crude Extract Electrophoresis Run. WR: broad-range molecular weight standard. M1, M2, M3, and M4: crude ligninolytic enzyme extracts obtained from three different *Pleurotus ostreatus* fermentation batches

bands in the range from 66 to 97 kDa. Most LACs from fungi have a molecular weight between 60 and 70 kDa [17]. LACs from *P. ostreatus* show molecular weights of 67.7 kDa, 60.4 kDa, and 18 kDa [43–45].

Pulp Extraction

Toquilla straw fibers used as a raw material to obtain pulp showed the following composition: moisture 8.88 ± 0.15%, lignin 20.10 ± 0.96%, and cellulose 33.29 ± 0.16%. After pulping, Kappa number was 66.46 ± 2.33, that corresponded to 8.64% of lignin. Pulp *freeness* was 591.25 ± 18.07, which is adequate for the enzyme – mediator system. Given that high freeness values, as is the case for these results, mean that fast drainage will allow easy access to lignin, thus the EMS was expected to operate efficiently [46].

Pulp Treatment with Enzyme-Mediator System

Table 1 presents the results of the kappa number for each treatment with respect to the control pulp, as well as the percentage of the residual lignin content and the percentage of delignification. The control pulp had an initial kappa number of 50.32 ± 1.95, with a lignin content of 6.54%, which was reduced due to washing with NaOH and H_2O_2 at the end of treatment, but not by enzymatic action. Hydrogen peroxide and sodium hydroxide act as delignifying agents, which remove small remaining organic compounds in the pulp and are used along with other compounds in TFC—totally chlorine free—bleaching techniques [47, 48].

When only ABTS was used (T1), the kappa number shows no statically difference from the control. Likewise, delignification difference from the control is 0.61%, therefore there is no effect due to chemical mediator alone. In contrast, treatments with enzyme alone (T2—T5) or enzyme and mediator (T6—T9) increased delignification. The highest delignification was achieved in EMS treatments (T6 – T9). Overall, EMS resulted in lower Kappa number, thus higher delignification of pulp among all treatments; T6 with 3.5 U/g of pulp and ABTS allowed for 19.72% more delignification compared to the Control. Some studies have demonstrated that increasing enzyme concentration also increases phenoxy radicals that combine among them, forming condensed lignin structures [49].

The use of ABTS as a mediator seems to increase the enzyme efficiency, in this case laccase with lignin phenolic and non-phenolic compounds. The EMS system has been widely studied [33–35, 50] when laccases from microorganisms in conjunction with ABTS as a mediator are used to delignify different types of wood pulp. Around 30% delignification is reached but no significant reduction is achieved when enzyme or mediator are use separately. The action mechanism of EMS is related to the oxidation of ABTS by ligninolytic enzymes, which then diffuses through secondary cellular wall and oxidizes both phenolic and non-phenolic lignin units. Subsequently, aromatic bonds are broken apart, while the mediator returns to its reduced original form [21, 37, 51]. Balakshin et al. [22] explain that the partial delignification of the pulp is due to the accumulation of lignin oxidized fragments highly reactive and diffusion limitation of chemicals agents, therefore not all remaining lignin can be removed but only a small amount. Better results would be expected if EMS is applied in consecutive steps on the same pulp [52].

Delignified toquilla straw pulp color depends on treatment applied as shown in Table 2. While the use of ABTS (T1) or enzyme alone (T2—T5) resulted in pulp showing luminosity and chroma (a* and b*) values like the control, and negative a* values representing a green hue, EMS treatments (T6—T9) produced positive a* values that correspond

Table 2 Color properties of toquilla straw pulp (*Carludovica palmata*) after enzymatic treatment with and without chemical mediator ABTS

Treatment	Brightness	a*	b*
Control	79.49 ± 1.79^{a}	-0.75 ± 0.07^{c}	$+23.53 \pm 0.74^{a}$
T1	78.36 ± 1.85^{a}	-0.95 ± 0.17^{c}	$+22.82 \pm 0.81^{a}$
T2	79.80 ± 0.98^{a}	-1.04 ± 2.44^{c}	$+23.23 \pm 1.20^{a}$
T3	78.80 ± 2.41^{a}	-0.90 ± 0.20^{c}	$+23.74 \pm 0.50^{a}$
T4	76.11 ± 4.12^{ab}	-0.70 ± 0.28^{c}	$+23.55 \pm 0.77^{a}$
T5	79.45 ± 1.48^{a}	-0.85 ± 0.20^{c}	$+23.49 \pm 0.69^{a}$
T6	72.14 ± 1.52^{cd}	$+4.08 \pm 0.99^{ab}$	$+18.78 \pm 2.17^{bc}$
T7	70.56 ± 4.24^{cd}	$+5.12 \pm 2.59^{a}$	$+16.17 \pm 6.16^{c}$
T8	72.91 ± 3.39^{bcd}	$+2.96 \pm 0.50^{b}$	$+22.21 \pm 1.70^{a}$
T9	73.85 ± 3.01^{bc}	$+2.83 \pm 0.23^{b}$	$+23.67 \pm 1.01^{a}$

Color properties are expressed as $mean \pm sd$ $(n = 4)$; different superscript letters denote significant differences (LSD, 95% confidence)

Table 1 Kappa number, lignin, and delignification percentage obtained from toquilla straw pulp (*Carludovica palmata*) after enzymatic treatment with and without chemical mediator ABTS

Treatment	Enzyme concentration U/g of pulp	ABTS mM	Kappa number	% Lignin	% Delignification
Control	0	0	50.32 ± 1.95^{d}	6.54	---
T1	0	1	49.98 ± 2.39^{d}	6.50	0.61
T2	3.5	0	45.19 ± 1.04^{bc}	5.87	10.24
T3	5	0	45.76 ± 0.88^{bc}	5.95	9.02
T4	10	0	46.03 ± 1.48^{c}	5.98	8.56
T5	20	0	46.59 ± 2.09^{c}	6.06	7.34
T6	3.5	1	40.41 ± 1.61^{a}	5.25	19.72
T7	5	1	42.04 ± 2.36^{a}	5.47	16.36
T8	10	1	43.13 ± 3.21^{ab}	5.61	14.22
T9	20	1	41.46 ± 3.11^{a}	5.39	17.58

Kappa values are expressed as $mean \pm sd$ $(n = 8)$; different superscript letters denote significant differences (LSD, 95% confidence)

to red hues with lower luminosity. These changes could be either due to the intense blue coloration on reaction medium caused by the ABTS oxidation by enzymes which dyed the pulp [53], or because of chromophore groups produced due to changes in pulp structure by EMS system [36]. Therefore, the EMS system has a negative effect on the color of delignified pulp. Coloration, nonetheless, can be modified by a bleaching step with alkaline solutions after the EMS application, which also helps to decrease organochlorine effluent while preserving the cellulosic structure [54–57].

Optimal Treatment Assessment

Surface response statistical analysis determined that an enzyme concentration of 14 U/ g pulp and 1 mM ABTS would be the optimal EMS conditions for toquilla straw pulp delignification. Characterization of toquilla straw pulp treated under these theoretical optimal conditions and a control (no EMS system) is detailed in Table 3. Compared to the control, the EMS produced pulp with a lower Kappa number, thus less lignin, and less luminosity which means a darker pulp than desired. However, a higher cellulose concentration was observed, which is a positive attribute because it means there was higher lignin degradation by the enzymatic-mediator action [58].

Finally, Table 4 shows the characterization of paper sheets made from pulp treated under the optimal EMS conditions, pulp treated under control conditions without enzyme or mediator (PC), and natural—cooked pulp without any additional treatment (PN); these last two were used as controls. Grammage varied among pulps due to initial weight adjustment to comply with TAPPI norms. Sheets made with EMS pulp had an explosion index 14.76% higher than control sheets. This can be attributed to the increase in carboxylic groups, which promotes the swelling and fibrillation of the fibers and in turn, improves the strength in fiber binding [58, 59]. The M/K Index provides an idea of homogenous fiber distribution; a lower number compared to the other sheets means a less uniform distribution, and potential defects, in this case possibly due to very short fibers that cause agglutination or so-called *flocs* [60]. Thickness depends on the grammage of paper sheets, having a higher grammage also caused greater consistency in their fibers, so the thickness of the sheets from the EMS pulp was also greater.

The resistance to air passage in the EMS treatment is not statistically different from the control, but with respect to the pulp without any treatment, this is also possibly due to the presence of fine fibers, which are intertwined in the sheet. The presence of fine fibrous materials promotes the formation of fiber–fiber bonds that consolidate the paper structure and promotes increased density, resulting in decreased air permeability of the paper [61].

Table 3 Characterization of toquilla straw pulp treated with 14 U/g pulp and 1 mM ABTS

	EMS	Control
Kappa Number	38.77 ± 0.086^{a}	50.32 ± 1.95^{b}
Lignin (%)	5.04^{a}	6.54^{b}
Cellulose (%)	76.36 ± 0.075^{a}	75.47 ± 0.056^{b}
Luminosity	70.92 ± 0.329^{a}	74.82 ± 0.445^{b}
±a	$+3.43 \pm 0.43^{a}$	-0.71 ± 0.3686^{b}
±b	$+21.11 \pm 1.61^{a}$	$+22.70 \pm 0.91^{a}$

Values are expressed as $mean \pm (n = 2)$; different superscript letters denote significant differences (LSD, 95% confidence)

Conclusions

Enzyme extracts from fermentation of *Pleurotus ostreatus* showed laccase activity (AE) up to 1261.11 U/L, with molecular weights ranging from 66 to 97 kDa.

The enzyme – mediator system (EMS) had a significant effect on decreasing Kappa number or residual toquilla straw pulp lignin, thus on cellulose purification; however, shows a negative effect in pulp luminosity since pulp is darker than control. Additionally, delignification treatment done with enzyme or ABTS alone had not significative effect.

The response-surface method determined 14 U/ g pulp and 1 mM ABTS as the optimum EMS system condition for Toquilla straw pulp delignification process, which reached a 5.04% lignin content.

Table 4 Physical properties of paper sheets made with three types of toquilla straw pulp

L	Grammage (g/m^2)	Explosion resistance index ($KPa*m^2/g$)	Resistance to air passage (s)	Thickness (mm)	M/K INDEX
PN	77.81 ± 1.89	1.77 ± 0.21^{a}	2.53 ± 0.39^{a}	0.20 ± 0.010^{a}	7.95 ± 1.48^{a}
PC	96.826 ± 1.21	1.78 ± 0.18^{a}	3.53 ± 0.38^{b}	0.23 ± 0.005^{b}	9.60 ± 1.27^{a}
EMS	113.785 ± 2.88	2.04 ± 0.21^{b}	3.67 ± 0.33^{b}	0.25 ± 0.021^{c}	5.65 ± 1.20^{a}

Values are expressed as $mean \pm sd$ $(n = 2)$; different superscript letters denote significant differences (LSD, 95% confidence)

Paper sheets made with EMS pulp increase their explosion index in 15%, but no significative differences were observed on the other properties.

Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Katherine Martínez y Gabriela Hidrobo. The first draft of the manuscript was written in Spanish by Katherine Martínez and Gabriela Hidrobo, translation was done by Gabriela Hidrobo and Neyda Espín all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets generated during and/or analyzed during the current study are not publicly available due to Escuela Politécnica Nacional does have access to a public data repository but are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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