Cellulose Fiber Enzymatic Modification to Improve the Softness, Strength, and Absorption Properties of Tissue Papers

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Effects of enzymatic modification were evaluated in bleached *Eucalyptus* kraft and sulfite cellulosic pulps, separately, to improve key tissue paper properties and design new *Eucalyptus* fiber applications. Different cellulase dosages (0.01 mg and 0.1 mg of enzyme/g of pulp) and reaction times (30 min and 60 min) were used to modify the fibers and replace the traditional mechanical based refining or beating process. The results showed that for enzymatic modified kraft and sulfite pulps, the softness properties were improved by 1 and 2 units, respectively, for each unit of decreased strength properties. To achieve a balance between the tissue properties, the different fiber pulp furnishes that contained 80% of the enzymatically treated kraft pulp and 20% of the sulfite pulp with and without enzymatic treatment, were studied. Overall, the structures made with these mixtures presented softness properties in the commercial range (57.8 to 74.4), improved absorption properties (107 mm to 120 mm of capillary rise), and good strength properties (13.0 to 17.7 N.m/g). This study was conducted in order to adjust the fiber furnishes according to industrial tissue standards, using one *Eucalyptus* fiber type providing strength and another providing softness.

Keywords: Biorefining; Enzymatic treatment; Eucalyptus pulps; Softness; Tensile Strength; Tissue Materials; Water Absorption

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INTRODUCTION

In recent years, the tissue paper industry has shown great growth potential. The abundance of raw materials, increased hygiene awareness, rising health spending, and better living standards have driven the global growth of this market. Tissue papers, such as toilet paper, towel paper, napkins, and facial tissues, are lightweight creped papers (Raunio *et al.* 2018). The fibers used in tissue paper manufacturing are predominantly hardwoods (60% to 100%), with a small percentage of softwoods (0 to 40%). *Eucalyptus* fibers have adequate dimensions and properties to develop tissue paper softness, absorption, and bulk (Pinto *et al.* 2005; Pirralho *et al.* 2014). Studies have shown that different *Eucalyptus* species presented different fiber dimensions that can be targeted to enhance specific paper properties (Dutt and Tyagi 2011; Pirralho *et al.* 2014; Morais *et al.* 2019). However, softwood fibers are necessary to confer strength properties to tissue products, which are important for the runnability of the paper machine (de Assis *et al.* 2018). Depending on the type of tissue paper, there will be a specific formulation with specific percentages of

hardwood and softwood fibers. However, in order to enhance fiber suitability and establish a more resistant three-dimensional matrix, the fibers are also subjected to fiber modification processes. These processes have potential to achieve diverse applications of *Eucalyptus* hardwoods in the tissue papers manufactured with optimized properties. Replacing softwood fibers with enzyme-modified *Eucalyptus* fibers can also be advantageous, allowing manufacturers to achieve the desired quality of these tissue materials and optimize the furnish management.

Refining is a mechanical treatment that improves the fiber quality by modifying its structure and morphology (Chauhan *et al.* 2011; Mou *et al.* 2013; Gharehkhani *et al.* 2015; Bajpai 2018). In general, the papermaking pulp refining process consists of applying compression and shear forces to the fibers in order to develop flexibility and ability to form inter-fiber bonds. This mechanical process also promotes fiber internal fibrillation, external fibrillation, and cutting (Bajpai 2018). The refining process increases the wet tensile strength properties of tissue papers and decreases their absorption properties (Gigac and Fišerová 2008). However, many efforts are currently underway to conserve energy during the refining process by treating pulps more efficiently with enzymes (Gharehkhani *et al.* 2015). Several industrial studies have shown that the use of different enzymes significantly reduced the refining energy necessary to achieve the required specifications for tissue papers, such as increased fiber bulk, tensile strength, and softness. Any treatment that promotes lower energy consumption will have a positive impact on global energy consumption in the pulp and tissue paper industries (Pathak *et al.* 2016).

In recent decades, the development of enzymatic technologies in the pulp and paper industry has increased. As a green and sustainable technology, enzymatic treatments have been studied extensively in different applications, such as improving the dissolving properties of pulps and reducing the chemicals used (Yang *et al.* 2019). The enzymes increase the fiber flexibility and pulp carboxylic group content, improve the handsheet density and smoothness, promote water absorption, swelling, fibrillation, and improve the tensile strength and burst strength properties (Jeffries 1992; Kenealy and Jeffries 2003; Lin *et al.* 2018). Nevertheless, an enzyme excess may reduce the pulp strength and erode the fiber surface (Kenealy and Jeffries 2003).

The influence of enzymes such as cellulase and xylanase or a mixture of these has been widely studied by several authors. The action of cellulases before refining has been shown to increase the pulp drainage, the fiber hydration degree, the handsheet mechanical properties and cell wall fiber surface degradation, and the bonding area of the water molecules (Wong *et al.* 2000; Gil *et al.* 2009; Efrati *et al.* 2013). The use of xylanases can produce refining energy savings and increase wet-fiber flexibility. Consequently, this can improve the inter-fiber bonding capacity by slowly decreasing the relative bonding area, improving optical brightness, and improving the physical strength properties (Spiridon *et al.* 2003; Spiridon and Duarte 2004; Buzała *et al.* 2016; Dutta *et al.* 2020). Even without mechanical refining, enzymatic treatment with the mixing of both enzymes results in modified pulp properties, such as increased tear strength, clear defibrillation, reduced fiber length, and increased fines (Žnidaršič-Plazl *et al.* 2009; Kumar *et al.* 2018). To the best of our knowledge, the application of enzymes in unrefined *Eucalyptus* pulp, with the goal to optimize tissue properties in order to produce a paper material with only *Eucalyptus* fibers has not been thoroughly investigated in the literature.

This study evaluated the effect of a treatment with a commercial enzyme in two *Eucalyptus* pulps. Different pulp mixtures with different treatments were still performed in order to optimize tissue properties, which increased the potential applications for the

Eucalyptus fiber. The effects of this enzyme and the mixtures were evaluated at different dosages and reaction times in terms of morphological, chemical and water interaction (swelling and pulp drainability) properties, as well as the softness, tensile strength, and absorption structure properties.

EXPERIMENTAL

Pulp Sample

An elemental chlorine-free (ECF) bleached *Eucalyptus globulus* kraft pulp and a total chlorine-free (TCF) bleached *Eucalyptus globulus* sulfite pulp were used in this study. These pulps were selected from a previous study (Morais *et al.* 2019, 2020) because the kraft pulp presented the best strength properties and the sulfite pulp presented the best softness properties, without any fiber modification treatment.

Enzyme Sample

The enzymatic treatments were carried out with an enzymatic preparation provided by a Portuguese company, consisting of the cellulase enzyme, with a density of 1.02 g/mL and an enzymatic activity of 2.2 filter-paper units (FPU)/mL.

Enzymatic Activity

The enzymatic activity of the commercial enzymatic preparation was determined according to the filter paper method, an International Union of Pure and Applied Chemistry (IUPAC) method (Ghose 1987; Yu *et al.* 2016). Whatman No. 1 filter paper (1.0 cm × 6.0 cm strips, with 50.0 mg ± 0.5 mg) was the substrate used in this procedure. Initially, 1 mL of 0.05 M Na-citrate buffer with a pH of 4.8 and filter paper strips were added to the test tubes. In order to saturate the filter paper strips, the test tubes were placed in a 50 °C bath for approximately 10 min. Then, 0.5 mL of diluted enzyme preparation was added to the tubes. Nine dilutions of the enzyme preparation were prepared. The tubes were incubated at 50 °C for exactly 60 min. After being removed from the bath, 3.0 mL of dinitrosalicylic acid (DNS) was added to each tube to stop the reaction. All the test tubes were placed in a boiling water bath for 5 min, followed by an ice water bath for 5 min. Finally, 20.0 mL of distilled water was added to each test tube. The prepared blanks (B1: 1.5 mL of citrate buffer; B2: 1.0 mL of citrate buffer + 0.5 mL of diluted enzyme preparation; B3: 1.5 of citrate buffer + filter paper strip) were treated the same as the above samples. The absorbance at 540 nm was measured using an ultraviolet (UV) spectrophotometer. Through the glucose calibration curve, it was possible to quantify the sugars released during the assay. The enzymatic activity was calculated to be FPU = 0.37 / cellulase concentration to release 2.0 mg glucose.

Methods

Enzymatic treatments

The pulps were treated with 0.01 mg and 0.1 mg of enzyme/g of pulp. These enzyme concentrations were selected because they correspond to extreme values, within the range of the standardized tissue industrial process. These treatments were carried out with pulp suspensions that had a pH of 7 and a consistency of 4% at 40 °C, with continuous mechanical agitation. An impeller was used to ensure the mass transfer effectiveness. Reaction times of 30 min and 1 h were tested. After the enzyme treatment, a minimum

amount of sodium hypochlorite was added to the pulp samples to stop the pulp enzyme reaction. Table 1 summarizes the assays performed.

Table 1. Enzymatic Treatment Assays Performed for the Kraft and Sulfite Pulps

Assay	Kraft Pulp	Assay	Sulfite Pulp
1	Without Treatment	6	Without Treatment
2	0.01 mg/g, 30 min	7	0.01 mg/g, 30 min
3	0.01 mg/g, 1 h	8	0.01 mg/g, 1 h
4	0.1 mg/g, 30 min	9	0.1 mg/g, 30 min
5	0.1 mg/g, 1 h	10	0.1 mg/g, 1 h

Pulp mixtures

After studying the enzyme-treated kraft and sulfite pulps, an experimental design was performed with different fiber pulp furnishes, containing 80% of the kraft *Eucalyptus* pulp enzymatically treated and 20% of the *Eucalyptus* sulfite pulp with and without enzymatic treatment (Table 2). The first four assays were carried out with the combination of kraft pulp with the different enzymatic treatments and sulfite pulp without any treatment. The last four assays were performed with the combination of kraft and sulfite pulps subjected to different enzymatic treatments.

Table 2. Mixtures of 80:20% Performed for the Enzymatically Treated Kraft and Sulfite Pulps

Assay	Treatments	Assay	Treatments
1	80% Assay **2**: 20% Assay **6**	5	80% Assay **2**: 20% Assay **7**
2	80% Assay **3**: 20% Assay **6**	6	80% Assay **3**: 20% Assay **8**
3	80% Assay **4**: 20% Assay **6**	7	80% Assay **4**: 20% Assay **9**
4	80% Assay **5**: 20% Assay **6**	8	80% Assay **5**: 20% Assay **10**

Pulp characterization

The fiber morphological properties were determined automatically by image analysis of a diluted suspension (20 mg/L) in a flow chamber, using the MorFi LB01 Fiber Size Analyzer (Techpap, Grenoble, France). The assay was performed in triplicate.

To understand the changes in the pulp functional groups, Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) was performed (Thermo Scientific, Nicolet IS 10 model, Waltham, Massachusetts, EUA). The FTIR-ATR technique made it possible to characterize, identify and quantify the efficiency of the enzymatic treatment in the kraft and sulfite pulps, through the presence or not of physical inter-fiber bonds. The handsheets surfaces produced with pulps without enzymatic modification (assays 1 and 6) and with application of maximum enzyme dosage and reaction time (assays 5 and 10) were analyzed. The samples were analyzed at a wavelength of 600 cm^{-1} to 4000 cm^{-1}, with a resolution of 4 cm^{-1}.

The Schopper-Riegler degree (°SR) method was determined, in triplicate, according to the ISO standard 5267-1 (1999) to measure the pulp drainability.

The water retention value (WRV) was determined according to the method described by Silvy *et al.* (1968), which is used to evaluate the fiber hydration. The wet pulp samples were centrifuged at a speed of 5,000 revolutions per min (4,640 g Force) for 10 min, using a centrifuge Kn-70 model, Kubota (Osaka, Japan). Then, the samples were removed, weighed, and oven-dried at 105 ± 2 °C for 24 h. The samples were again weighed.

The assay was performed in triplicate, and the WRV index (%) was calculated according to Eq. 1,

$$WRV = \frac{M1-M2}{M2} \times 100 \quad (1)$$

where *M1* is the mass of the wet sample after centrifugation, and *M2* is the mass of wet sample after drying at 105 °C.

Isotropic laboratory handsheet production and characterization

The laboratory handsheets with a basis weight approximately of 20 g/m^2 (tissue paper simulation in the laboratory) were prepared according to an adaptation of the ISO standard 5269-1 (2005). The pressing process was suppressed for the 20 g/m^2 handsheets. The structures were conditioned in a room at 23 ± 1 °C with a relative humidity of 50 ± 2%.

The scanning electron microscopic (SEM) images were obtained using a Hitachi S-2700 (Tokyo, Japan). Initially, the samples were coated with gold using a Sputter Quorum Q 15 OR ES (Laughton, East Sussex, UK) and analyzed with an accelerating voltage of 20 kV at different magnifications.

The basis weight was measured according to the ISO standard 12625-6 (2016). The thickness and bulk were determined according to the ISO standard 12625-3 (2016). A FRANK-PTI micrometer was used for thickness analysis. It is also important to mention that industrial tissue paper standards related to the tissue vocabulary (ISO:12625-1:ed-3:2019) cancels and replaces the second edition (ISO 12625-1:2011), which has been technically revised. The solids volume of handsheets was determined using a helium-gas displacement pycnometer (AccuPyc II), in a 10 cm^{-3} sample chamber. Through these volumes, the porosity (%) was calculated according to Eq. 2:

$$Porosity = \frac{Volume\ Total - Volume\ Solids}{Volume\ Total} \times 100 \quad (2)$$

The tensile index was determined according to the ISO standard 12625-4 (2016). The softness was analyzed using a Tissue Softness Analyzer (TSA) (Emtec, Leipzig, Germany). This analyzer uses different algorithms in order to calculate parameters related to the tissue paper softness, such as handfeel index (HF) and TS7 (parameter related to the surface softness from the fibers and the paper material) (Mendes *et al.* 2020). The algorithms selection depends on the sample analyzed, therefore the QAI algorithm was selected for the handsheets. For this analysis, five readings were made. The capillary rise was also determined by the ISO standard 8787 (1986).

Statistical Analysis

All data analysis was performed using Microsoft Excel Office 365, and statistical analysis was performed using independent samples t-test with a 95% confidence level with IBM SPSS Statistics 25 (Armonk, NY, USA).

RESULTS AND DISCUSSION

Kraft and Sulfite Pulps Enzymatic Treatments

The fiber morphological properties of the enzymatically treated pulps are shown in Table 3. The weighted fiber length and the weighted fiber width decreased for both the

pulps. The fiber lengths of the kraft and sulfite pulps decreased by 1.6% and 1.3%, respectively, for the maximum enzyme dosage and reaction time conditions compared to the pulps without enzymatic treatment. After the enzymatic treatment, the fiber widths ranged from 18.8 μm to 19.1 μm for the kraft pulp, and 18.3 μm to 18.9 μm for the sulfite pulp, which corresponded to a decreased fiber width of 1.6% and 0%, respectively. This slight decrease in the fiber length and width indicated that these two dimensions were not notably affected by the enzyme treatment. These results are in line with the higher fines content of 7.8% and 5.5% for the kraft and sulfite pulps, respectively, and the higher macrofibrils contents of 4.3% and 4.9% for the kraft and sulfite pulps, respectively. The coarseness of the sulfite pulp was approximately 2.75 times higher than the sulfite pulp (9.1% vs 3.3%).

Table 3. Effect of Enzymatic Treatments on the Pulp Morphological, Drainability, and Swelling Properties

Assays*	Weighted Length (mm)	Width (μm)	Coarseness (mg/100m)	Length of Macrofibrils (%)	Fines Elements (% in Length)	°SR	WRV (%)
1	0.798±0.004	19.1±0.1	6.83±0.10	0.509±0.005	37.1±0.3	20±0	76.3±2.6
2	0.797±0.002	18.8±0.1	6.76±0.09	0.526±0.027	37.4±0.1	21±0	89.7±2.8
3	0.795±0.001	18.9±0.1	6.64±0.18	0.526±0.010	38.4±0.6	22±1	90.3±3.1
4	0.792±0.002	18.8±0.1	6.48±0.17	0.529±0.009	39.2±1.0	26±0	92.3±2.4
5	0.785±0.003	18.8±0.1	6.21±0.10	0.531±0.017	40.0±0.3	28±1	92.5±1.7
6	0.696±0.002	18.3±0.1	7.26±0.28	0.588±0.018	42.0±1.6	18±0	63.1±1.8
7	0.696±0.004	18.9±0.1	7.17±0.25	0.600±0.030	42.2±0.5	19±1	73.9±4.3
8	0.690±0.002	18.6±0.0	7.16±0.24	0.603±0.044	42.2±0.4	20±0	77.8±2.7
9	0.690±0.001	18.5±0.1	7.06±0.14	0.616±0.015	42.8±1.9	22±1	79.9±3.1
10	0.687±0.003	18.4±0.1	7.02±0.63	0.617±0.011	44.3±0.3	23±0	80.1±3.1

Values reported are the mean ± standard deviations.
*Conditions specified in Table 1. Assays 1-5 correspond to kraft pulp and assays 6-10 correspond to sulfite pulp. Without treatment: assays 1 and 6. With 0.01 mg/g for 30 minutes (assays 2 and 7) and 60 minutes (assays 3 and 8). With 0.1 mg/g for 30 minutes (assays 4 and 9) and 60 minutes (assays 5 and 10).

Table 3 also shows the °SR and WRV properties of the enzyme-treated pulps. The biorefining process increased the drainage degree by 40% and 28% for the kraft pulp and the sulfite pulp, respectively, which is in line with the proportional increase in the pulp fines content. The enzymatic refining contributed to the increased fiber flexibility, which promoted the accessibility of the water molecules to the fiber structure (Spiridon *et al.* 2003). The °SR values were in the range of values used in the industrial tissue process (22 °SR to 28 °SR). The same drainage properties (22 °SR) were obtained for the kraft and sulfite pulps using different process conditions. The enzyme was dosed at 0.01 mg/g with 1 h of reaction time for the kraft pulp and 0.1 mg/g with 30 min of reaction time for the sulfite pulp. The WRV of the sulfite pulp was slightly higher than that of the kraft pulp. The WRV for the sulfite pulp increased by 27%, while the WRV for the kraft pulp increased by 21%. In this case, internal fibrillation and limitation of the enzyme penetration in the fiber wall were related to the WRV index increase (Gil *et al.* 2009; Gu *et al.* 2018), which facilitated higher fiber interaction and water retention.

To complement this study, the FTIR-ATR technique was used to identify some differences in the functional groups, with and without the enzymatic treatment applied to

the pulps. Figure 1 shows the FTIR spectrum of assays 1 and 5 for the kraft pulp and assays 6 and 10 for the sulfite pulp. The differences found in the spectrum were also supported by studies by other authors (Sridevi *et al.* 2016; Kumar *et al.* 2018; Dutta *et al.* 2020). The bands observed at the different wavelengths corresponded to the structural changes, as well as the cooking process used in the production of both pulps. The broad band at 3,000 cm^{-1} to 3,400 cm^{-1} indicated that the -OH stretching of the hydrogen-bonding and the band at 2,870 cm^{-1} to 3,000 cm^{-1} indicated that the =C-H stretching of the methyl group of cellulose and hemicelluloses. The band at 2,358 cm^{-1} to 2,360 cm^{-1} was due to the OH asymmetrical stretching vibration of the carboxylic acid in enzymatically treated pulps. The bands at 1,650 cm^{-1} to 1,652 cm^{-1} and at 1,202 cm^{-1} were due to the C=O stretch and the C-O stretch of hemicelluloses, respectively. It was still possible to verify that the bands at 1,428 cm^{-1}, 1,315 cm^{-1}, and 1,160 cm^{-1} indicated the C=H stretching, C=O stretch vibration in syringyl ring, and -COO- (carboxylate ion) group vibration, respectively.

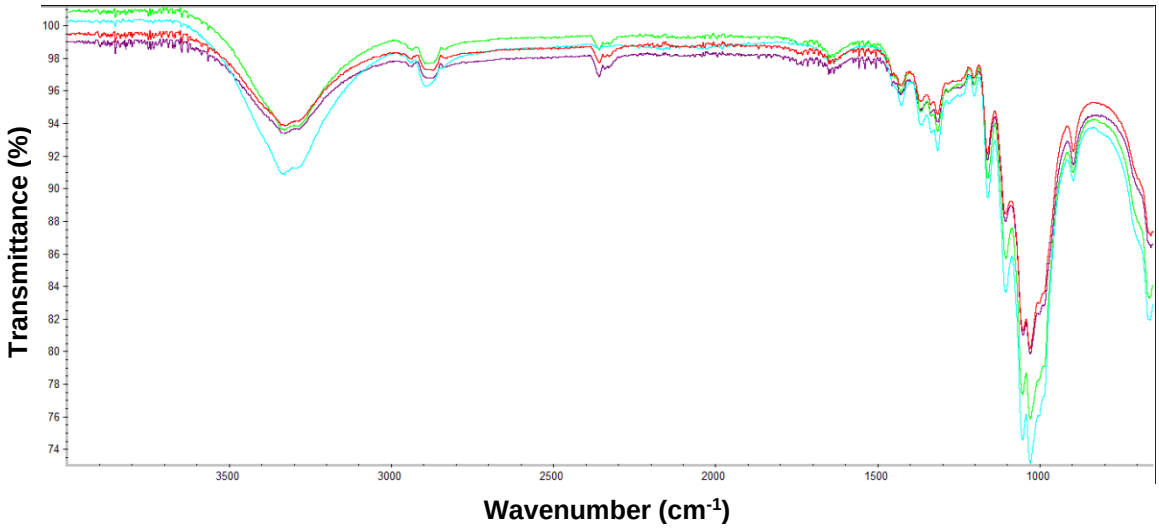

Fig. 1. The FTIR-ATR spectrum of the kraft pulp (assay 1, red) and sulfite pulp (assay 6, green) and the changes in these suspensions during the enzymatic treatment of assay 5 (purple) and assay 10 (blue) (0.1 mg/g, 1 h).

The SEM images (Fig. 2) also showed changes in the pulp fiber morphology after the enzymatic treatment. The enzymatic activity at the fiber surface level, which exposed external fibrillation, was observed for both pulps (Fig. 2b, d). The untreated pulps (Fig. 2a, c) also showed the presence of some fibrils, which was a result of the different cooking processes. Enzymes can hydrolyze the fines and cellulosic components accessible in the exposed fiber regions, smoothing their surface. In this enzyme dosage range, the bonds between fines and fibrils or between other fibers arise, modifying the morphological properties and drainage conditions (Table 3). This slight external fibrillation is also responsible for increasing the bonding points between fibers and, consequently, increasing the strength properties and decreasing the softness properties (Fig. 3).

This enzymatic degradation corresponded to changes in the tissue properties of the pulp structures, as shown in Fig. 3. The enzymatic action degrades the fiber wall, which forms macrofibrils and fines. Therefore, a more closed and compact structure formation with increases in the enzyme dosage and reaction time is expected. An increase in the enzymatic refining process caused a decrease in the handsheets porosity by 2.1% and 3.6%

for the kraft and sulfite pulps, respectively (Fig. 3a). This was a result of the increased relative bonding area between the fibers, which produced denser handsheets. Consequently, the bulk properties also decreased by 7% and 41% for the kraft and sulfite pulps, respectively (Fig. 3a).

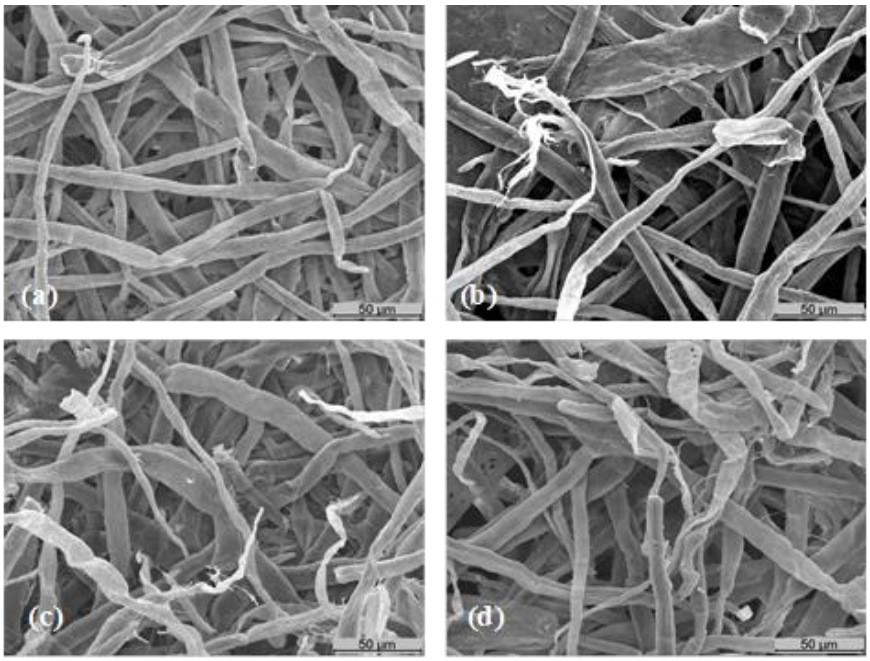

Fig. 2. The SEM images of a) untreated kraft pulp, b) treated kraft pulp from assay 5, c) untreated sulfite pulp, and d) treated sulfite pulp from assay 10. The treatment was an enzyme dosage of 0.1 mg/g and a reaction time of 1 h.

The softness HF, one of the essential properties for tissue papers, was negatively affected for both pulps over the enzyme dosage and reaction times (Fig. 3b). The decreased softness properties were promoted by the increase in the fiber flexibility, the fines content, and the inter-fiber bonding. The HF properties decreased by 29% and 14% for the kraft and sulfite pulps, respectively. The sulfite pulp samples had higher HF values (74.6 to 86.9) than the kraft pulp samples (45.1 to 63.2). This difference is due to the different production conditions for the pulps. The TS7 property is related to the material softness, in this case, the softness from the fibers present on the handsheet surface (Wang *et al.* 2019). The TSA equipment registered the noise that the ceramic blades produced when they passed across the sample surface. The more rigid fibers produced and registered more noise because the resistance of the fibers against the blades was higher. As the enzyme dosage and reaction time increased, the higher TS7 values were verified (Fig. 3b). This suggested that the enzymatically treated pulp fibers were more attached to the handsheet structure. The softness TS7 increased by 45% and 63% for the kraft pulp and sulfite pulp, respectively.

Unlike the softness HF, the tensile index was positively affected for both pulps throughout the enzyme dosage and reaction times (Fig. 3c). This parameter is important in tissue paper to ensure paper quality and paper machine runnability. The increased tensile index was a consequence of the enzymatic action, which promoted fibrillation by attacking the cellulosic fiber chains. This parameter increased by 166% for the kraft pulp and 371% for the sulfite pulp. The kraft pulp presented higher tensile indexes which agreed with the slightly lower porosities that were obtained.

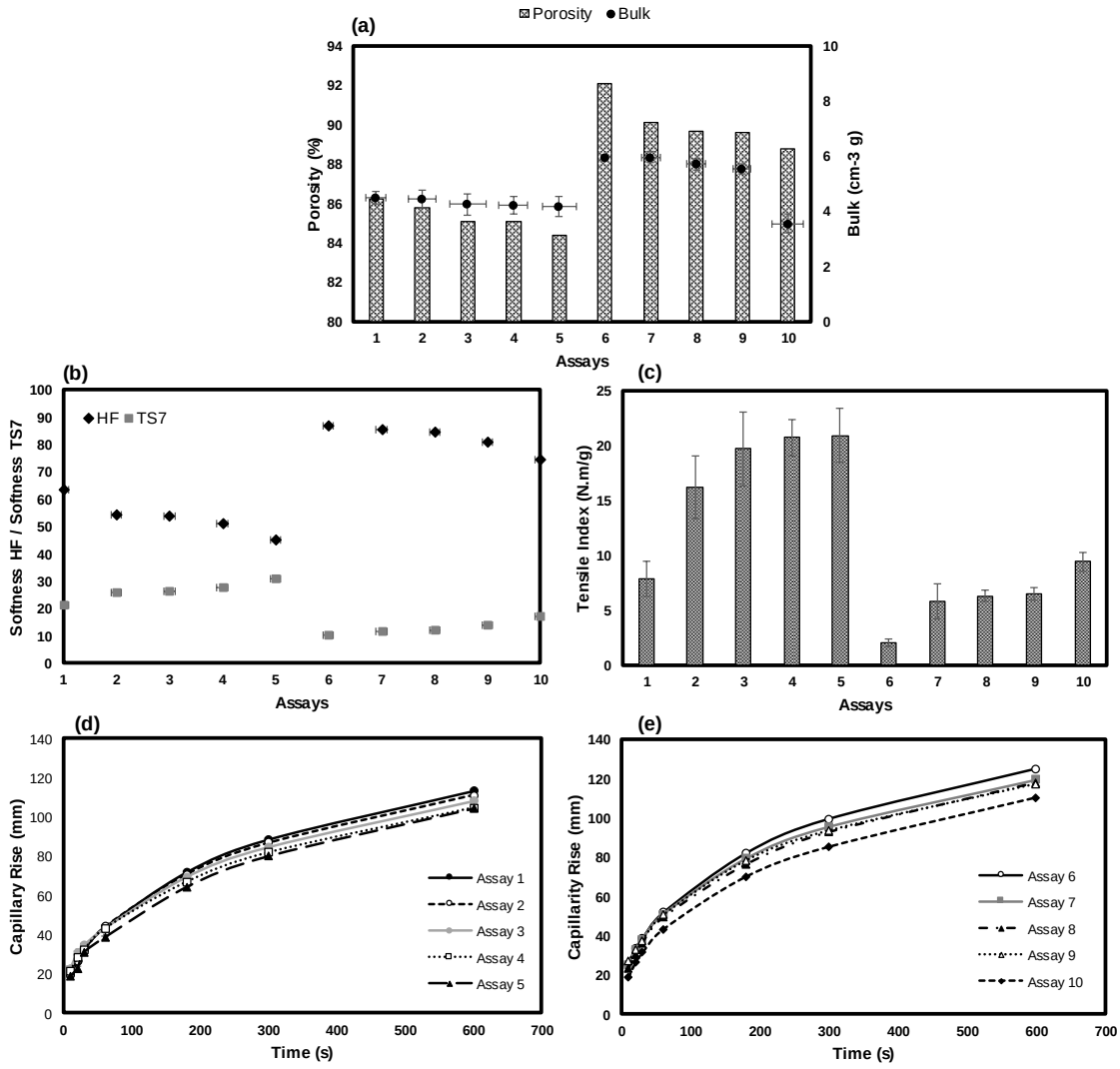

Fig. 3. The evolution of the tissue properties of the kraft and sulfite pulp handsheets during the enzymatic assays performed, namely, a) porosity and bulk, b) softness HF and TS7, c) tensile index, and the d,e) water capillary rise during enzymatic assays performed.

The tissue paper absorption properties depend on the porosity and fibers' hydrophilicity. Fiber fibrillation promoted a decrease in the water capillary rise in the structures. The kraft pulp presented values of capillary rise between 104 mm to 113 mm (Fig. 3d), while the sulfite pulp presented values between 110 mm to 125 mm (Fig. 3e). This trend was consistent with the softness HF values and the morphological properties. Decreases in the capillary rise at 10 min of 8% for kraft pulp and 12% for sulfite pulp were observed. However, despite these small differences found, the different levels of enzymatic treatment did not show significant differences in the capillary rise properties in both kraft and sulfite pulps ($t(5) = 0.55$, $p > 0.05$; $t(5) = 0.97$, $p > 0.05$). The critical value of student's t distribution with 5 degrees of freedom is 2.015, with a 95% confidence level.

With the different enzymatic treatments applied, the strength properties of the kraft pulp and the softness properties of the sulfite pulp were maintained. Therefore, it is important to select the process conditions that allow a good compromise between the key

tissue properties, in order to study the viability of producing tissue papers with different eucalyptus fiber pulps furnishes.

Pulp Mixtures Treatments

During the production of premium tissue papers, the optimization and management of furnish plays a key role in the resulting tissue paper materials characteristics. In order to adjust the key tissue properties, the next step of this work was to mix both pulps with the different enzyme treatments (Table 2) in order to evaluate the improvements in the tissue final end-use properties.

Table 4 presents the morphological and water interaction properties according to the studied mixtures. Comparing mixtures 1 and 2 (mixtures with 80% of kraft pulp treated with 0.01 mg/g and reaction times of 30 min and 60 min, respectively, and with 20% of sulfite pulp without enzymatic treatment), it was found that the weighted fiber length decreased by 0.27%, the coarseness decreased by 1.47%, the macrofibrils content increased by 1.29%, and the fines content increased by 4.40%. The drainage degree increased by 4.76% and the WRV increased by 13.18%. Regarding the analysis of mixtures 3 and 4 (mixtures with 80% of kraft pulp treated with 0.1 mg/g and reaction times of 30 and 60 min, respectively, and with 20% of sulfite pulp without enzymatic treatment), the weighted fiber length and the coarseness decreased in percentages similar to mixtures 1 and 2 (0.26% *vs.* 1.15%), the macrofibrils content increased by 2.90%, and the fines content only increased by 0.25%. Additionally, increases of 7.69% and 28.84% were observed in the °SR and WRV properties for these mixtures, respectively.

Table 4. Effect of Pulp Mixtures in the Pulp Morphological, Drainability, and Swelling Properties

Assays*	Weighted Length (mm)	Width (µm)	Coarseness (mg/100m)	Length of Macrofibrils (%)	Fines Elements (% in Length)	°SR	WRV (%)
1	0.780±0.000	19.0±0.1	6.82±0.02	0.543±0.013	36.4±0.6	21±0	85.0±2.1
2	0.778±0.002	19.0±0.1	6.72±0.19	0.550±0.010	38.0±0.3	22±0	96.2±2.6
3	0.775±0.002	18.7±0.1	6.08±0.09	0.551±0.008	40.1±1.3	26±1	141.1±6.1
4	0.773±0.001	18.5±0.5	6.01±0.19	0.567±0.017	40.2±0.9	28±0	181.8±2.1
5	0.777±0.001	19.0±0.1	6.77±0.04	0.520±0.002	37.6±0.5	20±1	144.0±5.1
6	0.776±0.002	18.8±0.1	6.75±0.16	0.532±0.009	38.9±0.1	21±0	180.8±1.2
7	0.776±0.002	18.7±0.1	6.73±0.05	0.537±0.003	39.4±0.4	27±1	204.3±4.0
8	0.769±0.002	18.7±0.1	6.48±0.13	0.553±0.015	39.6±0.9	28±0	291.0±4.1

Values reported are the mean ± standard deviations.
*Conditions specified in Table 2. Assays 1-4 correspond to mixtures of 80% enzyme-treated kraft pulp, with different enzyme dosage and reaction time conditions with 20% sulfite pulp without treatment. Assays 5-8 correspond to mixtures 80:20 of both enzyme-treated pulps under the same conditions.

Regarding mixtures 5 and 7 (mixtures of the kraft and sulfite pulps treated with an enzyme dosage of 0.01 mg/g and 0.1 mg/g, respectively, for 30 min), the fiber length decreased by 0.13%, the coarseness decreased by 0.59%, the macrofibrils content increased by 3.3%, and the fines content increased by 4.8%. The drainage properties by 35% and the WRV increased by 42%. For mixtures 6 and 8 (mixtures of kraft and sulfite pulps treated with an enzyme dosage of 0.01 mg/g and 0.1 mg/g, respectively, for 1 h), the fiber length decreased by 0.90%, the coarseness decreased by 4%, the macrofibrils content increased

by 3.9%, and the fines content increased by 1.8%. The drainage properties were found in the same range of mixtures 5 and 7 (33%), and the WRV increased by 61%.

Differences in the structure of the 3D networks were also observed in the pulp mixtures (Fig. 4). For example, mixture 4 showed fibrils on the fiber walls, exposing external fibrillation (Fig. 4a). In mixture 8, external fibrillation on the fiber walls as well as inter-fiber bonds were more visible (Fig. 4b).

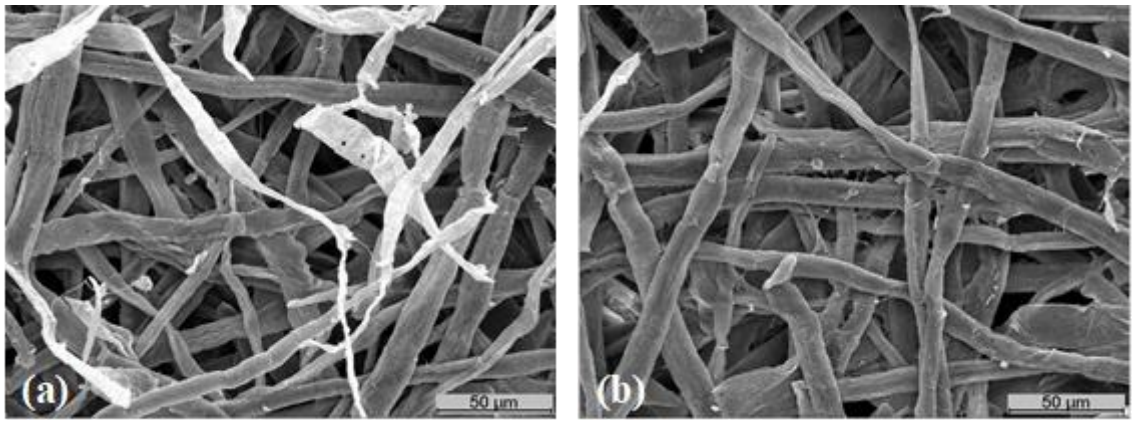

Fig. 4. The SEM images of a) pulp mixture 4 and b) pulp mixture 8

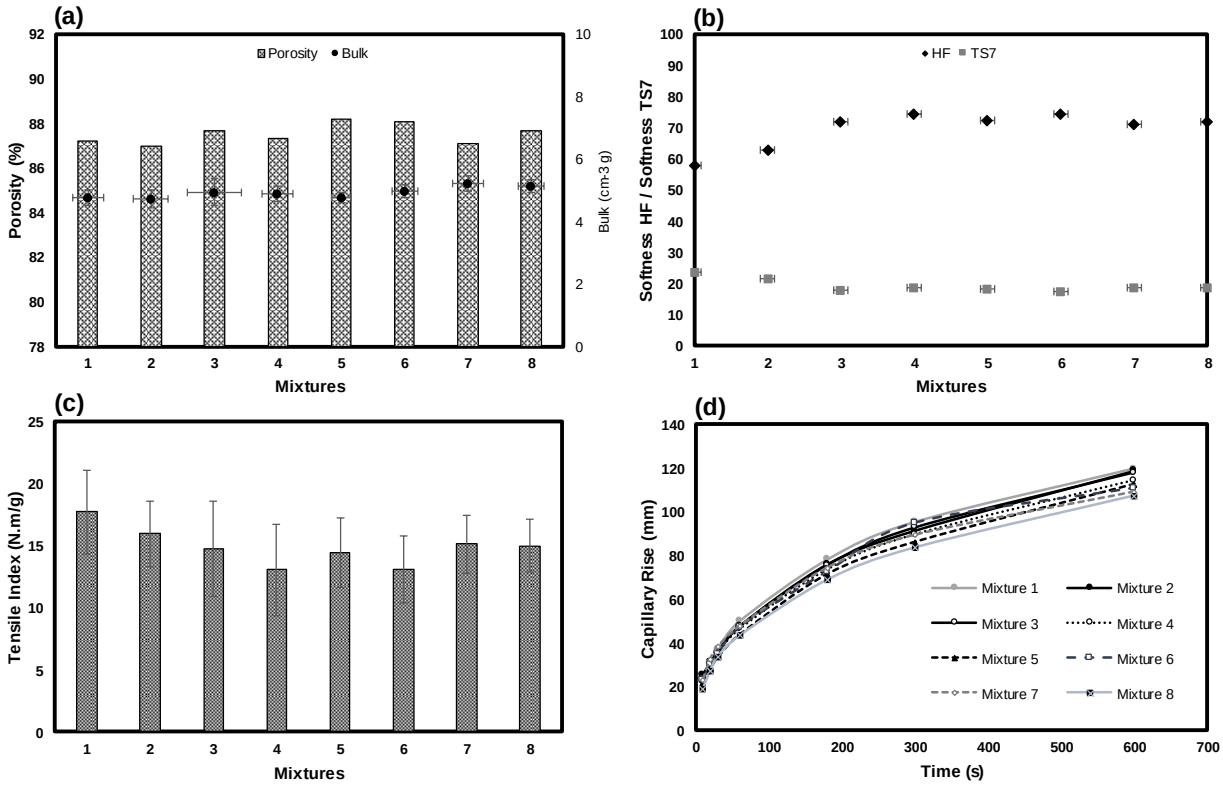

Fig. 5. The evolution of the tissue properties for the pulp mixtures, namely, a) porosity and bulk, b) softness HF and TS7, c) tensile index, and d) water capillary rise

Figure 5 presents the tissue properties of the handsheets produced with the studied mixtures. According to the structural properties, the pulp mixtures influenced the handsheet

porosity and the bulk (Fig. 5a). Very similar properties were observed. The porosity was between 87.0% and 88.2% and the bulk was between 4.72 cm^3/g and 5.21 cm^3/g.

The handsheet mixtures had a softness HF that ranged from 57.8 to 74.4 (Fig. 5b). Values in this range are found in commercial tissue papers. The inverse relationship between the softness HF and the TS7 was also verified. The tensile index of the handsheet mixtures showed values between 13.0 and 17.7 N.m/g (Fig. 5c). Stronger structures were produced with the different mixtures, along with good levels of softness, compared to the enzyme-treated pulps separately. Mixtures 4 and 6 presented the maximum values of the softness HF and minimum values of the tensile index. The compatibility of these two opposing properties will depend on the type of tissue paper to be produced.

Additionally, the inverse relationship of the softness HF and the tensile index properties were verified in both the separate and combined enzymatically treated pulps (Fig. 6). The relationship of these two variables in modified the kraft pulp, the sulfite pulp, and the pulp mixtures can be explained, respectively, by the models HF = −1.08 Tensile + 72.01 (R^2 = 0.820), HF = −1.62 Tensile + 92.19 (R^2 = 0.758), and HF = −3.68 Tensile + 124.36 (R^2 = 0.858). Previous studies reported that untreated hardwood pulps have also shown an inverse tendency for these two variables (Morais *et al.* 2019, 2020). For these untreated hardwood pulps, for every 2.4 units of increased softness, one unit of the tensile index was reduced. However, for the kraft pulp, the enzymatically modified sulfite pulp, and the pulp mixtures, for each 1.1, 1.6, and 3.7 units of increased softness, respectively, one unit of the tensile index was reduced.

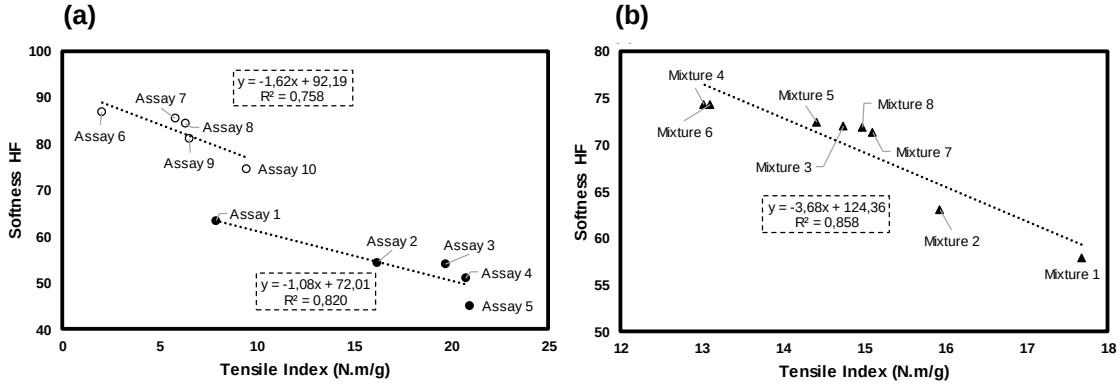

Fig. 6. Correlation of the tensile index and the softness HF for the a) enzymatically treated kraft and sulfite pulps and the b) pulp mixtures

Regarding the water absorption properties, the capillary rise of the handsheets were between 107 mm and 120 mm (Fig. 5d). Similar capillary rise profiles for the different mixtures were observed. The mixtures of both enzymatically treated pulps (mixtures 5 through 8) showed lower capillary rise values than the mixtures with the untreated sulfite pulp (mixtures 1 through 4). The structure capillarity decreased as the enzyme dosage and reaction time increased. However, despite these small differences found, the different levels of enzymatic treatment did not show significant differences in the capillary rise properties in eucalyptus fiber pulp mixture furnishes ($t(8) = 0.558$, $p > 0.05$). The critical value of student's t distribution with 8 degrees of freedom is 1.860, with a 95% confidence level.

A fundamental step in tissue paper production is the creping process. This process promotes paper structural changes when it is scraped from the Yankee cylinder by the action of a creping blade. This results in fiber orientation changes and paper creping. This

process contributes to elongation and softness properties, and therefore it is extremely important for the final quality of tissue papers (Raunio and Ritala 2012; Pan *et al.* 2019; Mendes *et al.* 2020). The present research work did not involve the creping process of the isotropic laboratory handsheets, so the prediction of the tissue functional properties did not include the creping step of tissue making. If the strategy presented in our work is feasible to be implemented industrially, it is important to understand how the creping process can influence the results. We believe that the creping process will decrease the mechanical properties of the tissue papers produced with the eucalyptus pulps maximization since the softness and strength properties are inversely related. From this perspective, we also believe that the investigation of the creping and embossing processes quantification in the softness, strength, and, also, absorption properties is necessary for advanced predictions.

CONCLUSIONS

1. The results achieved in this work showed that biorefining with enzymes contributed to changes in the tissue properties of kraft and sulfite industrial pulps. The enzymatic treatment of pulp contributed to more efficient fiber fibrillation, increased fines formation, and improved fiber bonding. Additionally, the individualized enzymatic treatment for both pulps promoted the maintenance of the strength and softness properties for the kraft and sulfite pulps, respectively, as verified in Fig. 3.
2. In order to balance the inverse relationship between softness and strength properties, different furnish combinations of enzymatically treated eucalyptus pulps were studied. The combination of the enzymatically treated kraft and sulfite eucalyptus pulps allowed the fiber furnish adjustment to develop the key properties of tissue paper materials. The softness, strength, and absorption properties of the low basis weight structures improved, compared to the pulp samples that were treated separately, as shown in Fig. 5.
3. Overall, this study identified the different conditions of the pulp mixtures that facilitate the best compromise between softness, strength and absorption properties for different types of tissue papers. For example, premium toilet or facial papers can be produced with pulps that have good softness properties, such as mixtures 4 and 6, and premium towel papers or napkins can be produced with pulps that have good strength and absorption properties, such as mixtures 1 and 2.

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