Mild Enzymatic Treatment of Bleached Pulp for Tissue Production

Magdalena Kmiotek,[a,*] Katarzyna Dybka-Stępień,[b] and Anna Karmazyn [a]

Effects of cellulase enzymatic treatment followed by mechanical beating were evaluated relative to the properties of cellulase-derived tissue pulps and handsheets. When different cellulase concentrations (0.0012 FPU/g, 0.0018 FPU/g, and 0.0024 FPU/g) of oven dried pulp (a 65/35 w/w ratio of beech to eucalyptus) were used for tissue production, a slight deterioration of the morphological characteristics was observed. Thus, a possibility of controlling the changes in the degree of polymerization of cellulose, as well as the fiber properties (in particular the length and coarseness) appeared. With an increased treatment time and enzyme concentration, these effects increased. The enzyme activity did not affect the apparent density of the paper, but the porosity drastically increased. The zero-span strength of the enzymatically treated pulps decreased with an increase in treatment time and amount of cellulase. However, mechanical beating improved the bonding between the cellulase fibers, which helped prevent the eventual decrease in mechanical properties of the handsheets. With the use of cellulase, the proposed moderate changes to fiber structure were achieved, giving the possibility of predicting and controlling the properties of tissue paper.

Keywords: Tissue pulp; Beech pulp; Eucalyptus pulp; Cellulase; Enzyme treatment; Mechanical beating; Morphology; Degree of polymerization; Mechanical strength

*Contact information: a: Centre of Papermaking and Printing, Lodz University of Technology, Wolczanska 223 Street, 90-924 Lodz, Poland; b: Institute of Fermentation Technology and Microbiology, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Wolczanska 171/173 Street, 90-924 Lodz, Poland; *Corresponding author: magdalena.kmiotek@p.lodz.pl*

INTRODUCTION

Tissue paper is one of the most popular paper types and has an ever-increasing rate of production. Over the last 30 years, the total global consumption of tissue paper has increased at a rate of approximately 1 million tons per year, which consumed approximately 40% of the total bleached chemical pulp shipments in 2016 (Bach 2018). Overall higher consumption of sanitary and household grade paper was observed to be accelerated by the SARS-CoV-2 pandemic (CEPI 2021). According to RISI (Resource Information System Inc. 2016), the total global tissue production should exceed 44 million tonnes in 2021, which constitutes a growth of over 14 million tons from 2010 (Sirois 2016). In 2020, hygienic paper constituted 9.3% of the total paper and board production in Europe (CEPI 2021).

The considerable diversification in the rate of annual consumption of tissue paper between Eastern and Western Europe (4% to 5% in comparison to 2%) was caused by socio-economic factors, *e.g.*, the rate of urbanization, increase in net income, and consumer spending (Bach 2018). From 2000 to 2014, the contribution of Eastern Europe (namely Poland and Russia) in total European hygienic paper consumption has increased from 12%

to 22% (Uutela 2016a). In Poland, the per capita consumption of tissue is relatively high (12 kg in 2015, which is close to the Western Europe average level of 16 kg) and shows signs of gradual development, especially for towels products (Uutela 2016b).

Tissue paper, namely paper for hygienic or domestic use, refers to paper handkerchiefs, tissues, kitchen paper, toilet paper, paper napkins, *etc.*, which are composed of 100% virgin (primarily hardwood kraft fiber), recycled (office paper collection program), or mixed cellulose fibers. In terms of key end-use tissue paper properties, absorbency, softness, and tear resistance are the most important (de Assis *et al.* 2018). The first two, which are primarily affected by the porosity, creping (surface property), and furnish composition of the paper (often accompanied by smoothness), are typical for chemical and semi-chemical pulps produced from various hardwoods (beech, birch, poplar, aspen, or eucalyptus). In order to obtain good mechanical strength, these pulps need to be treated with enzymes and mechanically beaten.

The application of enzymes to tissue paper production processes increases profitability, reduces the financial costs connected with energy consumption depletion, and provides adaptability to increasingly stringent environmental requirements (Kenealy and Jeffries 2003). The use of enzymes enabled Olli Jokinen of Genencor International Mill to increase paper machine speed by 7%, as well as to reduce the total energy consumption per ton of produced paper by 7.5% (Jokinen and Hagstrom-Nasi 1992). Enzymes can reduce the accumulation of adhesives and pitch residues, called stickies, on paper machines (Jokinen and Hagstrom-Nasi 1992; Gutierrez *et al*. 2001). During the production of sulphite and mechanical pulps from softwood (namely *Pinus sylvestris*), a high amount of resin is produced, which is responsible for pitch formation (Gutierrez *et al*. 2010). The application of lipases can control its accumulation during paper formation on the paper machine (Ballinas-Casarrubias *et al*. 2020). Enzymes are also applied to help remove contaminants in the recycle stream (Pathak *et al*. 2011; Liu *et al*. 2017).

Enzymes can be applied in environmentally friendly bleaching processes, which reduces or even eliminates the need of chlorine in elemental chlorine free (ECF) or totally chlorine free (TCF) bleaching processes. An enzyme technology based on xylanases has greatly reduced the amount of chlorinated aromatic by-products produced during pulp bleaching (Kenealy and Jeffries 2003; Salgueiro *et al*. 2016; Li *et al*. 2018).

Enzymes can facilitate the deinking of recycled paper and improve the drainage of pulps containing recycled fibers in the feedstock stream. The reduction of costs involved in deinking (Bajpai and Bajpai 1998) and higher drainage rates enabling faster paper machines operating speeds, have increased the income of manufacturers, which was possible due to enzymes utilization (Bajpai 1999; Singh *et al*. 2016; Saxena and Chauhan 2017). In addition, this has decreased the demand on timber resources (Kenealy and Jeffries 2003).

Enzymes can be used in the refining process of virgin fibers. Kraft pulps can be treated with cellulases and xylanases, which results in a reduction of total energy required to further refine the pulps (Dickson *et al*. 2000). Enzyme activation energies depend on the temperature, pH, time of treatment, and sequence with which the process is performed. Enzyme treatment before refining leads to a decrease in total energy required to meet the fiber strength specifications and can also contribute strength properties to the fiber at a fixed refining level (Lumiainen 2013). The addition of enzymes after refining leads to an increase in freeness, making the operation speed of the paper machine faster (Moran 1996). Enzyme-assisted refining can enable lower head box consistency, which results in better paper formation, improved strength, decreased basis weight, and an increased usability of

recycled fibers (Kenealy and Jeffries 2003; Lumiainen 2013; Gharehkhani *et al*. 2015). It should be mentioned that the use of cellulases in paper production needs to be carefully monitored, as such treatment will influence the strength of paper (Seo *et al*. 2000).

Either refining or mechanically beating of pulp is an effective method for increasing the connectivity of the cellulose nanostructures within the paper network. Refining results in the development of pulp fibers during the papermaking process, making it possible to reach the desired level of quality (Sain *et al*. 2002; Kang *et al*. 2006a,b). During the refining process, internal fibrillation, external fibrillation, fiber shortening, *i.e.*, cutting, and fines formation occur. Fibrillation is the peeling-off of the primary wall and S1 layer of the fiber wall, which uncovers and exposes the S2 layer, allowing it to form inter-fiber bonds (Sain *et al*. 2002; Kang *et al*. 2006a,b; Afra *et al*. 2013). The cellulosic fines that are formed from the delamination of the outer layers of the fibers tend to be slender and flexible, which improves the bonding properties of the paper (Sigoillot *et al*. 2001; Cui *et al*. 2015).

Enzyme-assisted refining can lead to an improvement in the drainability of pulp of up to 80%, while using the same amount of energy during the refining process or decrease the tear strength depending on the mixture used, the pH, and the type of cellulase (Oksanen *et al*. 1997; Garcia *et al*. 2002; Taipale *et al*. 2010; Lee *et al*. 2013). Many studies have investigated the biorefining process of pulp in terms of cellulases usage and their influence on the drainability and paper sheet properties. Cellulases are commercially used in tissue paper production to improve the softness and absorbance of the end product, which are among the most important tissue features. As shown in numerous studies, the application of enzymes does affect the strength of pulp, which is primarily due to fibrillation and fines element production (Gharehkhani *et al.* 2015).

However, there is no published evidence in terms of the biorefining process for blends of beech and eucalyptus kraft pulps and its effect on fiber morphological characteristics and paper strength. For this reason, the authors decided to apply cellulase enzyme to the biorefining process of pulp blend for usage in tissue paper production and investigate its effect on the polymerization degree of cellulose and the morphological parameters of fibers first treated with commercial enzyme and then mechanically beaten. Furthermore, the influence of the biorefining process on the properties of the paper sheets made from the refined pulp was measured. As such, the author's goal was to find a tool for shaping and controlling the properties of pulp and tissue paper *via* the optimization of the amount of cellulase and treatment time during the enzymatic treatment. According to our knowledge, such an approach has never been applied before and constitute the novelty of conducted research.

EXPERIMENTAL

Materials and Methods

Pulp samples

The kraft pulp investigated in this study was a blend of ECF bleached beech (*Fagus sylvatica* L.) and eucalyptus (*Eucalyptus grandis, E. dunnii, E. globulus, E. maidenii*) kraft pulps. Beech kraft pulp was purchased from Bukocel (Hencovce, Slovakia) and eucalyptus kraft pulp was obtained from UPM Euca (UPM, Helsinki, Finland). Beech and eucalyptus kraft pulps containing 5% (w/w) of moisture were mixed at ratio of 65 to 35 (by weight w/w), respectively.

Pulp blends were subjected to further treatment as illustrated in Fig. 1.

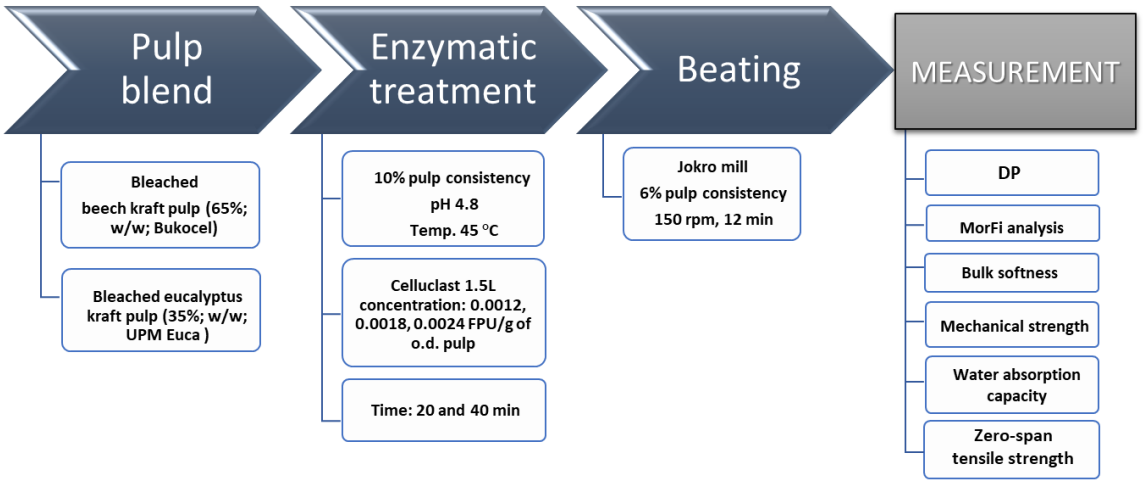

Fig. 1. The main stages of research

Enzyme activity

The cellulase enzyme used (Celluclast 1.5L) was purchased from Novozymes, Bagsværd, Denmark. Celluclast 1.5L is an aqueous solution of cellulase derived from *Trichoderma reesei* produced *via* submerged fermentation. This enzyme brand contains a broad spectrum of cellulolytic enzyme activities, notably cellobiohydrolases and endo-1,4-β-glucanases (belonging to hydrolases, which constitute the 3rd class of enzymes).

The enzyme activity (Filter Paper Unit, FPU) was determined by the method described by Ghose *et al.* (1987). In the previously mentioned procedure, the substrate was a 50 mg Whatman No. 1 filter paper strip (1.0 cm x 6.0 cm).

The detection of glycosidic bond cleavage *via* this approach involves the dinitrosalicylic acid method (DNS), which measures the amount of reducing sugars generated *via* enzymatic hydrolysis (absorption readings at 540 nm) (Miller 1959). The enzyme activity was measured at 50 °C and pH equal to 7, which was adjusted with diluted sulphuric acid.

Enzymatic treatment of pulp

Air dried pulp, consisting of 65% (w/w) beech pulp and 35% (w/w) eucalyptus pulp, was pre-soaked overnight and disintegrated for 5 min in laboratory high-speed universal disintegrator (Biobase HSD-80, Biobase Bioindustry, China) according to ISO 5263-1 (2004) standard. The kraft pulp samples were treated with commercial cellulase preparations in 1 L beakers at 10% pulp consistency, a pH of 4.8 (adjusted using diluted H_2SO_4), and a temperature of 45 °C for 20 min and 40 min with continuous mechanical agitation.

The enzyme solution was added to the final concentration of 0.0012 FPU/g, 0.0018 FPU/g, or 0.0024 FPU/g of o. d. (oven dried) pulp, as presented in Table 1. The control pulp (without enzymatic treatment) was included in the experiment in the way described above. The enzymatic reaction was stopped by bringing the mixture to a boil and incubation in the boiling water bath for 5 min, then quickly cooling down to room temperature in cold water bath and subjected to forming handsheets immediately. For each experiment, two simultaneous trials were carried out.

Table 1. Samples Description

	Sample Name						
	Control	A1	A2	B1	B2	C1	C2
Enzyme Dosage of Oven Dried Pulp (FPU/g)	0	0.0012	0.0012	0.0018	0.0018	0.0024	0.0024
Enzymatic Treatment Time (min)	0	20	40	20	40	20	40

Degree of cellulose polymerization

The degree of cellulose polymerization was measured according to the procedure described in TAPPI standard T 230 om-08 (2008). The air-dried pulp samples were dissolved in 25 mL of 0.5 M copper ethylenediamine (CED) for 10 min. The intrinsic viscosities of the solutions were obtained with an Ű bbelohde capillary viscometer, and these values were converted to degree of polymerization values *via* the Immergut−Schurz−Mark equation (Immergut *et al*. 1953), as shown in Eq. 1,

$$DP^{0.905} = 0.75 \times [\eta] \quad (1)$$

where $[\eta]$ is the intrinsic viscosity, and DP is the degree of polymerization.

Zero-span tensile strength

The fibre strength of the studied pulp samples was measured with a Pulmac Zero-Span Tester (Pulmac Systems International, Williston, VT) according to the procedure described in TAPPI standard T 231 cm-96 (1996). Paper handsheets with a 60 g/m^2 basis weight were cut into 15 mm width and 90 mm long specimens. After being watered with trays of water, the specimens were placed into an apparatus and a 0.2 mm span was applied.

MorFi analysis

The fiber length, width, coarseness, kink angle, kinked fibers amount, and other morphological indices of pulp, including the fine elements content, were measured using a MorFi LB-01 apparatus (TechPap Company, Gières, France), according to ISO standard 16065-2 (2014). The equipment was composed of measuring tackle and computer devices combined with the MorFi program, which allowed for the analysis of the tests results. In a water medium, 0.2 g of o. d. pulp was disintegrated, and was scanned by laser beam while flowing through a capillary tube.

Anatomical characteristics of pulps

Small, air-dried samples of the studied pulps were immersed in distilled water and brought to boil for 3 min to 5 min. A few droplets of the fiber suspensions were placed on microscopic glasses and dyed with Herzberg reagent. The studied pulp samples were viewed and photographed with an optical microscope (Biolar D, PZO Warsaw, Poland) with a 10 x / 0.25 dry objective, as well as being equipped with a camera (DMC 2900, Leica, Wetzlar, Germany) and an image analyzing system (Optika Vision Pro, Ponteranica, Italy).

Mechanical beating

The mechanical beating of the mixed beech and eucalyptus pulp (65% to 35% by

w/w, respectively) was performed in a laboratory Jokro mill at a pulp consistency of 6 wt. % in time evaluated on the basis of our previous studies (Danielewicz *et al.* 2015) in accordance with the PN-EN standard 25264-3 (1999). Jokro mill, similar to PFI mill, consists of several refining grooved rolls placed in containers and rotating at the same speed reaching 150 rpm. The groove dimensions in Jokro mill are smaller than PFI mill, especially in groove depth (20 mm versus 50 mm). The time is regulated for controlling the refining.

The properties of handsheets

Using a Rapid-Köthen apparatus, 75 g/m^2 ± 3 g/m^2 handsheets were prepared according to PN EN ISO 5269-2 (2007). The handsheets were conditioned in accordance with PN EN 20187 (2000) standard before further assessment of their properties. The drainability was determined according to PN EN ISO 5267-1 (2002). The brightness R457, basis weight, specific volume, air resistance, tensile, burst and tear strength of the samples were measured in accordance with the ISO 2470-1 (2008), ISO 536 (2012), ISO 534 (2011), ISO 5636-5 (2003), ISO 1924-2 (2008), ISO 2758 (2008), and ISO 21974 (2002) standards, respectively. The water absorption capacity was investigated based on basket-immersion method (ISO 12625-8, 2010).

RESULTS AND DISCUSSION

Treatment of the cellulase of the studied pulps led to a decrease in the degree of polymerization of the cellulose chain, (Table 2). The initial DP of the bleached kraft beech/eucalyptus pulp mix (65% to 35% by w/w, respectively) was 763. With an increased treatment duration and cellulase concentration, the DP decreased. Doubling the length of the enzyme reaction caused the DP to reduce by 2.5% to 3.5% in comparison to the DP obtained after a 20 min treatment. However, the reduction in the DP of cellulose was minimal, between 0.0018 FPU and 0.0024 FPU of applied enzyme per 1 g of o. d. pulp. It is worth noting that the enzymatic treatment with 0.0018 FPU/g for 20 min (pulp B1) had a comparable effect to the 40 min treatment with 0.0012 FPU of enzyme per 1 g of o.d. pulp (pulp A2).

Table 2. Degree of Polymerization of Cellulose in the Analysed Pulp Samples

Property	Sample						
	Control	A1	A2	B1	B2	C1	C2
[η] (cm^3/g)	542	539	522	525	513	520	483
DP	763 ± 1	759 ± 4	733 ± 3	738 ± 2	719 ± 2	729 ± 3	707 ± 3

For economic reasons, it is reasonable to question whether it is better to bear the costs of enzymes or energy expenditure. In this study, moderate shaping of the cellulose chain length using a small amount of enzymes and reaction lengths was achieved, rather than a sharp lowering of the DP as would be the intended target for biofuels production. The maximum DP reduction revealed in the study was on the level of *ca.* 7% (in relation

to initial DP), whereas with a 18 h long treatment, the DP was decreased by *ca.* 76%, as described by Valls *et al*. (2019). The aim of this research was not to obtain saccharides, but to achieve the knowledge needed for controlling the DP of cellulose *via* enzymatic treatment.

The morphological elements of the studied pulps were typical of hardwood pulps, *i.e.*, libriform/fibers (f), vessels (v), and parenchyma (p) cells. The photos documenting the morphology of the analysed pulps are presented in Fig. 2.

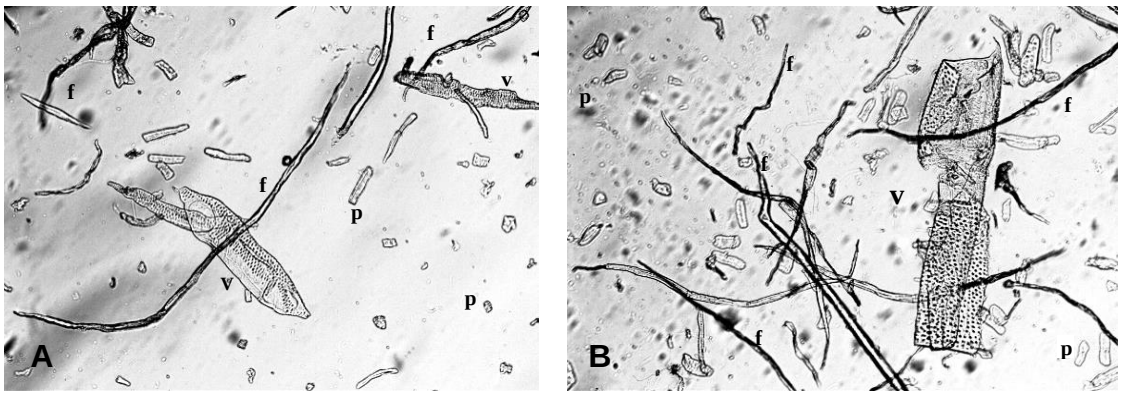

Fig. 2. The morphology of bleached (A) beech and (B) eucalyptus kraft pulp (40x magnification)

The beech pulp was characterized by the occurrence of middle-large vessel elements with simple perforations and scattered pitting patterns. In some vessel elements, short tails were placed on both vessel edges. In addition, scalariform perforations in the vessels also occurred, and vasicentric tracheids were occasionally present. The parenchymal cells were fairly narrow and long. The typical dimensions of the beech fibers were as follows: an average length of 1.2 mm (0.5 mm to 1.7 mm) and an average width of 21 μm (13 μm to 40 μm), whereas the average length of the eucalyptus fibers was 1.1 mm (0.3 mm to 1.5 mm) and the average width was 20 μm (10 μm to 28 μm). For eucalyptus pulp, it was characterized by the occurrence of long vessel elements with simple perforated plates and narrow threadlike tail(s) placed on one or both vessel edges. The vessel elements possessed characteristic ray parenchyma pitting. In addition, long and short vasicentric tracheids, as well as fiber tracheids, could be found in eucalyptus pulps (Ilvessalo-Pfäffli 1995).

The results of the morphological analysis of the studied pulp samples before and after enzymatic treatment with cellulase, are shown in Table 3, demonstrating the changes in fiber dimensions.

The lowest enzyme concentration and treatment length caused a gradual reduction of fiber width. The enzymatic treatment of studied pulp samples caused a slight thinning of the fiber width, especially when the highest enzyme concentration was applied, Table 3.

The longer the treatment time and the enzyme concentration, the shorter the fiber, *e.g.*, for pulp C2, there was a 5% reduction in length. For pulp samples treated with 0.0012 FPU of enzyme per 1 g of o. d. pulp, a fiber shortening effect was not observed. In the case of an enzyme concentration of 0.0024 FPU/g of o. d. pulp, the fibers were shortened with a longer enzyme treatment time, which was shown by a change in fibre length by approximately 5% in comparison to a 20 min enzyme treatment time.

Table 3. Morphological Characteristics of Pulps Treated with Cellulase

Pulp Samples	Properties						
	Control	A1	A2	B1	B2	C1	C2
Number of Fibers (10^6/g)	13.017	13.178	12.417	13.205	13.205	12.599	13.482
Fiber Length (weighted in length) (mm)	0.763	0.775	0.765	0.756	0.755	0.761	0.727
Fiber Width (μm)	21.5	21.5	21.5	21.4	21.5	21.3	21.4
Fines Content (% in area)	15.03	15.95	16.09	15.84	15.59	15.00	16.62
Coarseness (mg/m)	0.1264	0.1243	0.1184	0.1199	0.1205	0.1144	0.1119
Kinked Fibers (%)	18.8	18.4	17.9	17.8	17.1	16.5	16.2
Curl fibers (%)	6.1	6.0	5.9	5.9	5.8	5.6	5.5

These observations corresponded well with the changes in DP (Table 2). According to Wang *et al.* (2019), hardwood fibers with a short length produced more free fiber ends on the surface, which ultimately resulted in an increased softness feeling on the surface of tissue product. With an increase in enzymatic treatment parameters, the fines content of the studied pulp samples increased as well (Table 3), which was an effect of the enzymatic hydrolysis of cellulose chains by endoglucanases, which were able to randomly cleave the β-1,4-glycosidic linkages in the cellulose fibers. As a result, at the same time cellodextrine fragments, soluble in water, and shortened cellulose chains are formed (Caspi *et al*. 2011). The more advanced the process, the more likely the formation of fine elements. For example, in the case of C1 and C2 samples, the increased time of enzyme action led to a higher fine elements content, as shown in Table 3. On the other hand, such a tendency cannot be seen in the case A1, B1, C1 or A2, B2, C2 samples are compared.

In applied conditions, the higher the enzyme concentration and treatment time, the better the coarseness values of the pulp samples, in terms of usage as a tissue paper (Table 3). With lower coarseness values, a higher handfeel, higher tensile strength at a constant number of fibers, higher absorption and bulk, and better formation occurred (Ramezani and Nazhad 2004). There tended to be a reduction in coarseness with shorter fiber fractions, which was observed in the current study. The longer duration of enzymatic reaction and cellulase concentration, the lower the kinked and curled fibers content in the pulps, Table 3. The curled fibers present in beaten pulps produce papers with lower tensile strength and tensile stiffness. The same is observed in the case of kinked fibers (Page *et al.* 1979). The observed decrease in amount of kinked and curled fibers (Table 3) with increasing enzyme concentration and time of its action should result in the improved mechanical properties of the studied papers.

The next step of the research was evaluating the mechanical beating process of the enzymatically treated beech/eucalyptus pulp under conditions given in the Experimental section and papersheets formation coming next.

Table 4. Schopper-Riegler (°SR) degree, Brightness, Specific Volume, Bulk Softness, Water Absorption Capacity, and Air Resistance, of Studied Pulps Treated with Cellulase

Pulp	Drainability (°SR)	Brightness (%)	Specific Volume (cm^3/g)	Bulk Softness (kNm^{-1})	Water Absorption Capacity (g/g)	Air Resistance (s/100 cm^3 of air)
Control	31 ± 0.5	69.69 ± 0.53	0.623 ± 0.09	286.2 ± 8.7	2.40 ± 0.27	21.09 ± 0.71
A1	28 ± 1.5	70.70 ± 0.45	0.629 ± 0.07	283.1 ± 10.1	2.46 ± 0.23	25.66 ± 0.28
A2	26 ± 1	68.77 ± 0.45	0.627 ± 0.10	307.9 ± 18.3	2.61 ± 0.28	24.58 ± 0.37
B1	27.5 ± 0.5	71.30 ± 0.21	0.610 ± 0.15	310.1 ± 9.7	2.62 ± 0.27	14.35 ± 0.66
B2	25 ± 0.5	71.06 ± 0.48	0.630 ± 0.02	305.8 ± 11.7	2.65 ± 0.27	15.46 ± 0.71
C1	24 ± 1	70.15 ± 0.21	0.623 ± 0.09	368.9 ± 12.1	2.68 ± 0.26	14.56 ± 0.41
C2	24 ± 0.5	70.72 ± 0.50	0.624 ± 0.06	355.8 ± 24.7	2.76 ± 0.29	12.88 ± 0.53

The drainability decreased with the enzymatic treatment time and enzyme concentration increasing (Table 4; *e.g.*, compare samples A1 and A2, as well as A1, B1, and C1). There was not a major difference between the values obtained for the different pulp samples, with the drainability of the non-treated pulp reaching up to 22.6%. Such a decrease in drainability should not noticeably affect the drainage of the wire section on a paper machine.

Enzymatic treatment of the pulp slightly increased its brightness (Table 4). Such an increase is within the limits of error and should not be considered as an effect of the enzymatic treatment. For all studied pulp samples, the specific volume did not change, which suggested that absorptivity was not adversely affected.

For hygienic papers, softness and absorbency are of high interest. Softness can be distinguished as so-called bulk softness, which is the perception of softness when a sample is crumpled between the hands and surface softness and obtained when fingerprints are lightly brushed over the sample surface (Gigac 2008). In this research, bulk softness was evaluated based on tensional stiffness (Table 4). It can be estimated that after enzymatic action, the bulk softness is higher. The higher its concentration, the higher the softness. The duration of enzymatic reaction does not influence the softness. This means that softness of studied pulps is rather strongly dependent on the enzyme concentration rather than on the time of its action.

Absorbency of studied samples was measured as water absorption capacity (Table 4). Enzymatic treatment of the studied pulps with their subsequent mechanical beating slightly influenced the amount of water absorbed by 1 g of the pulps. However, with increasing enzyme concentration, the water absorption capacity increased (compare A1, B1, C1 and A2, B2, C2). The same was observed with longer time of enzymatic reaction (see A1, A2; B1, B2 and C1, C2).

A difference in the air resistance of the pulp samples could be seen, which was determined by the Gurley method. This indicated that the tested samples had different porosity values. More porous paper was obtained when the lowest enzyme concentration

was applied. For the 0.0018 FPU and 0.0024 FPU of cellulase per 1 g of o. d. pulp samples, the air resistance decreased from the initial time of air permeation (21.09 s) to the range from 12.88 s to 15.46 s, which indicated the formation of a much more porous structure in comparison to the paper not treated with enzyme. The results of the air resistance testing corresponded to the freeness of the studied pulp samples; lower values were observed with a higher freeness, which was an effect of a higher coarse fiber content (Table 3) (Hubbe 2001).

The zero-span tensile strength determined for the pulp samples subjected to mechanical beating after being treated with cellulase revealed minor deterioration of the fiber strength (Fig. 3). Notably, the zero-span tensile strength decreased along with increased enzyme concentration (compare results for sample Control, A1, B1, C1 and Control, A2, B2, C2). However, at the same time, it was noted that the longer enzyme treatment time had less effect on the zero-span tensile strength in comparison to the shorter time. This was due to the enzymatic cleavage of the 1,4-β-glycosidic bonds between the structural repeating units in the cellulose chain as well as the shortening of fiber length, which is detrimental to the strength properties of fibers and paper.

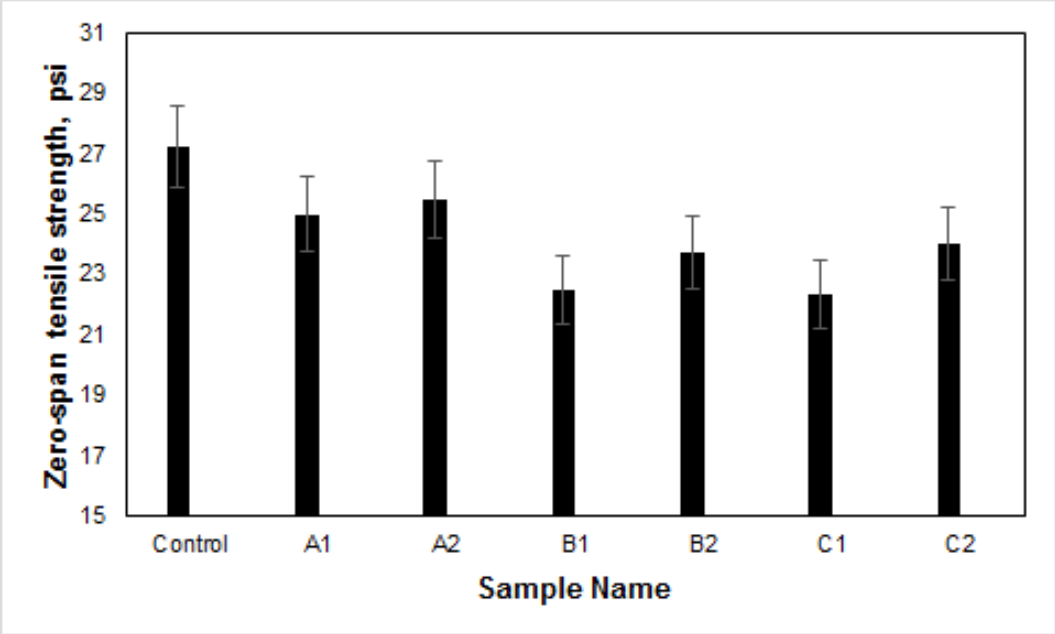

Fig. 3. The zero-span tensile strength of the handsheets from studied pulp samples treated with cellulase

The fiber strength values (Table 5; Fig. 4) corresponded well with the DP of pulp samples (Table 2). However, the decrease in fiber strength did not exceed 20%, even with the highest enzyme concentration. The lowest decrease in fiber strength was 6.5%, which was observed in pulp samples A1 and A2 (reflecting the lowest enzyme amount). This could indicate that determining and controlling the intended DP and fiber strength would be possible by properly setting amount of enzyme to a known enzymatic activity.

Table 5. Properties of Handsheets from Studied Pulps

Pulp Samples	Stretch (%)	Tensile Index (N·m/g)	Burst Index (kPa·m²/g)	Tear Index (mN·m²/g)
Control	2.82 ± 0.30	52.30 ± 2.74	3.24 ± 0.05	7.56 ± 1.65
A1	3.11 ± 0.20	49,81 ± 1.40	3.03 ± 0.22	7.12 ± 0.34
A2	2.92 ± 0.24	53.32 ± 2.82	2.76 ± 0.54	7.46 ± 0.52
B1	2.49 ± 0.23	49.86 ± 2.85	2.67 ± 0.23	6.25 ± 0.76
B2	2.77 ± 0.19	52.68 ± 2.79	3.09 ± 0.08	7.26 ± 1.41
C1	2.65 ± 0.27	49.92 ± 1.92	2.84 ± 0.18	7.13 ± 1.67
C2	2.32 ± 0.16	54.35 ± 2.28	2.94 ± 0.39	6.68 ± 0.70

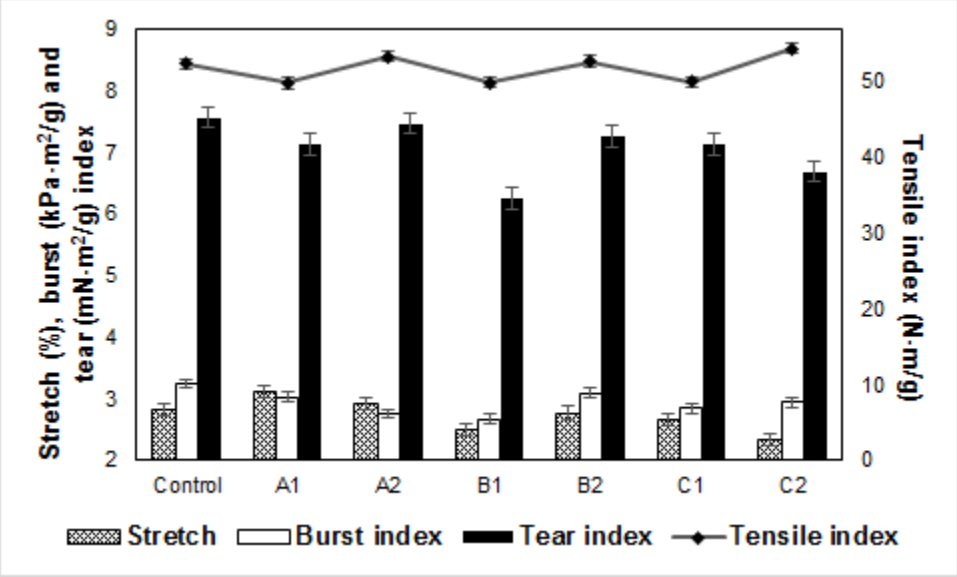

Fig. 4. Mechanical properties (stretch, burst index, tear index and tensile index) of handsheets from studied pulps

An enzymatic pre-treatment of pulp under mild conditions (low enzyme concentration and enzyme application time) followed by mechanical beating did not have a major effect on the stretch, burst, and tear index of the pulp samples. Little difference was found between the obtained values and were within the error limits. Regardless of the enzyme concentration used, the tensile index value was maintained at the same level for both shorter and longer time of the enzymatic treatment of the pulp. However, the observed fluctuation between them was not relevant (8% relatively), which showed a lack of dependence on the enzyme concentration and exposure time in terms of its enzymatic activity

CONCLUSIONS

1. The results obtained indicated the possibility of determining and controlling the intended degree of polymerization (DP) and fiber strength of the pulp sample by properly setting the amount of enzyme, with a known enzymatic activity rate, and the total treatment time.

2. The application of cellulase leads to a slight deterioration of fiber strength of the studied pulp samples, which is typical for this enzyme (Kim *et al*. 2006; Gil *et al*. 2009; Liu *et al*. 2012).
3. The deterioration in fiber strength corresponded well with the changes in cellulose chain length (DP) and morphological characteristics of the pulp fibers (fiber length and coarseness).
4. As the cooperation of enzymes in the refining process are already known (Spiridon *et al.* 2001; Pelletier *et al*. 2013), the application of cellulase can be a valuable way to obtain a moderate change in handsheet properties, depending on the enzyme concentration and treatment time.
5. The fiber strength changes are reflected in the tensile and tear index values, which were measured for the studied pulp sample handsheets.
6. The changes in burst index values were minute when the samples were subjected to the enzyme concentrations and treatment times in this study.

REFERENCES CITED

Afra, E., Yousefi, H., Hadilam, M. M., and Nishino, T. (2013). "Comparative effect of mechanical beating and nanofibrillation of cellulose on paper properties made from bagasse and softwood pulps," *Carbohydrate Polymers* 97(2), 725-730. DOI: 10.1016/j.carbpol.2013.05.032

Bach, P. (2018). "Market pulp: Tissue has become the biggest global buyer," (https://www.tissuestory.com/2018/04/26/market-pulp-tissue-has-become-the-biggest-global-buyer/), Accessed January 15, 2020.

Bajpai, P. (1999). "Application of enzymes in the pulp and paper industry," *Biotechnology Progress* 15(2), 147-157. DOI: 10.1021/bp990013k

Bajpai, P., and Bajpai, P. K. (1998). "Deinking with enzymes: A review," *TAPPI Journal* 81(12), 111-117.

Ballinas-Casarrubias, L., González-Sánchez, G., Eguiarte-Franco, S., Siqueiros-Cendón, T., Flores-Gallardo, S., Duarte Villa, E., de Dios Hernandez, M., Rocha-Gutiérrez, B., and Rascón-Cruz, Q. (2020). "Chemical characterization and enzymatic control of stickies in kraft paper production," *Polymers* 12, 245.

Caspi, R. (2011). "MetaCyc Pathway: Cellulose degradation II (fungi)," MetaCyc database (https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=PWY-6788), Accessed February 3, 2020.

CEPI (2021). "PreliminaryStatistics 2020" (https://www.cepi.org/wp-content/uploads/2021/02/Preliminary-Draft2020.pdf), Accessed February 23, 2021.

Cui, L., Meddeb-Mouelhi, F., Laframboise, F., and Beauregard, M. (2015). "Effect of commercial cellulases and refining on kraft pulp properties: Correlations between treatment impacts and enzymatic activity components," *Carbohydrate Polymers* 115, 193-199. DOI: 10.1016/j.carbpol.2014.08.076

Danielewicz, D., Surma-Ślusarska, B., Żurek, G., Martyniak, D., Kmiotek, M., and Dybka-Stępień, K. (2015). "Selected grass plants as biomass fuels and raw materials for papermaking, Part II. Pulp and paper properties," *BioResources* 10(4), 8552-8564.

de Assis, T., Reisinger, L. W., Pal, L., Pawlak, J., Jameel, H., and Gonzalez, R. W. (2018). "Understanding the effect of machine technology and cellulosic fibers on tissue properties – A Review," *BioResources* 13(2), 4593-4629.

Dickson, A. R., Wong, K. K. Y., and Mansfield, S. D. (2000). "Response of xylanase-treated kraft pulp to Escher-Wyss and PFI refining," *TAPPI Journal* 83(7), 64-75.

García, O., Torres, A. L., Colom, J. F., Pastor, F. I. J., Díaz, P., and Vidal, T. (2002). "Effect of cellulase-assisted refining on the properties of dried and never-dried eucalyptus pulp," *Cellulose* 9(2), 115-125. DOI: 10.1023/A:1020191622764

Gharehkhani, S., Sadeghinezhad, E., Kazi, S. N., Yarmand, H., Badarudin, A., Safaei, M. R., and Zubir M .N. M. (2015). "Basic effects of pulp refining on fiber properties – A review," *Carbohydrate Polymers* 115, 785-803. DOI: 10.1016/j.carbpol.2014.08.047

Ghose, T. K., Montenecourt, B. S., and Eveleigh, D. E. (1987). "Measurement of cellulase activities," *Pure and Applied Chemistry* 59(2), 257-268. DOI: 10.1351/pac198759020257

Gigac, J., and Fiserova, M. (2008). "Influence of pulp refining on tissue paper properties," *Tappi Journal* 7(8), 27-32.

Gil, N., Gil, C., Amaral, M. E., Costa, A. P., and Duarte, A. P. (2009). "Use of enzymes to improve the refining of a bleached *Eucalyptus globulus* kraft pulp," *Biochemical Engineering Journal* 46(2), 89-95. DOI: 10.1016/j.bej.2009.04.011

Gutiérrez, A., del Río, J. C., and Martínez, A. T. (2010). "Fungi and their enzymes for pitch control in the pulp and paper industry," in: *Industrial Applications*, 2nd Edition, M. Hofrichter (ed.), Springer-Verlag, Berlin, Germany, pp. 357-377.

Gutierrez, A., del Río, J. C., Martínez, M. J., and Martínez, A. T. (2001). "The biotechnological control of pitch in paper pulp manufacturing," *Trends in Biotechnology* 19(9), 340-348. DOI: 10.1016/S0167-7799(01)01705-X

Hubbe, M. (2001). "Porosity (Too permeable, not permeable enough)," (https://projects.ncsu.edu/project/hubbepaperchem/TShoot/G_Poros.htm), Accessed April 10, 2020.

Ilvessalo-Pfäffli, M. S. (1995). *Fiber Atlas: Identification of Papermaking Fibers*, Springer Verlag, Berlin, Germany.

Immergut, E. H., Schurz, J., and Mark, H. (1953) "Viskositätszahl-molekulargewichts-beziehung für Cellulose und Untersuchungen von Nitrocellulose in verschiedenen Lösungsmitteln [Viscosity number-molecular weight relationship for cellulose and studies of nitrocellulose in various solvents]," *Monatshefte fuer Chemie* [Chemical Monthly] 84, 219-249. DOI: 10.1007/BF00899186

ISO 534 (2011). "Paper and board – Determination of thickness, density and specific volume," International Organization for Standardization, Geneva, Switzerland.

ISO 536 (2012). "Paper and board – Determination of grammage," International Organization for Standardization, Geneva, Switzerland.

ISO 12625-8 (2010). "Tissue paper and tissue products – Water-absorption and water-absorption capacity, basket-immersion test method," International Organization for Standardization, Geneva, Switzerland.

ISO 1924-2 (2008). "Paper and board – Determination of tensile properties – Part 2: Constant rate of elongation method (20 mm/min)," International Organization for Standardization, Geneva, Switzerland.

ISO 2470-1 (2008). "Paper, board and pulps – Measurement of diffuse blue reflectance factor – Part 2: Outdoor daylight conditions (D65 brightness)," International Organization for Standardization, Geneva, Switzerland.

ISO 2758 (2008). "Paper – Determination of bursting strength," International Organization for Standardization, Geneva, Switzerland.
ISO 5263-1 (2004). "Pulps – Laboratory wet disintegration – Part 1: Disintegration of chemical pulp," International Organization for Standardization, Geneva, Switzerland.
ISO 5636-5 (2003). "Paper and board – Determination of air permeance and air resistance (medium range) – Part 5: Gurley method," International Organization for Standardization, Geneva, Switzerland.
ISO 16065-2 (2014). "Pulps – Determination of fibre length by automated optical analysis – Part 2: Unpolarized light method," International Organization for Standardization, Geneva, Switzerland.
ISO 21974 (2002). "Paper – Determination of tearing resistance (Elmendorf method)," International Organization for Standardization, Geneva, Switzerland.
Jokinen, O., and Hagstrom-Nasi, C. (1992). "A method for reducing pitch trouble in mechanical pulp," Publication No. WO/1992/016687.
Kang, T., and Paulapuro, H. (2006a). "Effect of external fibrillation on paper strength," *Pulp and Paper Canada* 107(7), 51-54.
Kang, T., and Paulapuro, H. (2006b). "New mechanical treatment for chemical pulp," *Journal of Process Mechanical Engineering* 220(3), 161-166. DOI: 10.1243/09544089JPME81
Kenealy, W. R., and Jeffries, T. W. (2003). "Enzyme processes for pulp and paper: A review of recent developments," in: *Wood Deterioration and Preservation*, B. Goodell, D. D. Nicholas, and T. P. Schultz (ed.), American Chemical Society, Washington, D.C., U. S., pp. 210-239.
Kim, H.-J., Jo, B.-M., and Lee, S.-H. (2006). "Potential for energy saving in refining of cellulase-treated kraft pulp," *Journal of Industrial and Engineering Chemistry* 12(4), 578-583.
Lee, C. K., Ibrahim, D., and Omar, I. C. (2013). "Enzymatic deinking of various types of wastepaper: Efficiency and characteristics," *Process Biochemistry* 48(2), 299-305. DOI: 10.1016/j.procbio.2012.12.015
Li, P., Hou, Q., Zhang, M., and Li, X. (2018). "Environmentally friendly bleaching on bamboo (*Neosinocalamus*) kraft pulp cooked by displacement digester system," *BioResources* 13(1), 450-461. DOI: 10.15376/biores.13.1.450-461
Liu, N., Qin, M., Gao, Y., Li, Z., Fu, Y., and Xu, Q. (2012). "Pulp properties and fiber characteristics of xylanase-treated aspen APMP," *BioResources* 7(3), 3367-3377. DOI: 10.15376/biores.7.3.3367-3377
Liu, M., Yang, S., Long, L., Wu, S., and Ding, S. (2017). "The enzymatic deinking of waste papers by engineered bifunctional chimeric neutral lipase – endoglucanase," *BioResources* 12(3), 6812-6831
Lumiainen, J. (2013). "Refining of chemical pulp in papermaking part 1, sock prepration and wet end," (http://turbulence-initiated.sites.olt.ubc.ca/files/2013/01/1998-Lumiainen-Ch4.pdf), Accessed April 10, 2020.
Moran, B. R. (1996). "Enzyme treatment improves refining efficiency, recycled fiber freeness," *Pulp and Paper* 70(9), 119-121.
Oksanen, T., Pere, J., Buchert, J., and Viikari, L. (1997). "The effect of *Trichoderma reesei* cellulases and hemicellulases on the paper technical properties of never-dried bleached kraft pulp," *Cellulose* 4, 329-339. DOI: 10.1023/A:1018456411031
Page, D. H., Seth, R. S., and De Grace, J. H. (1979). "The elastic modulus of paper I. The controlling mechanisms" *Tappi* 62, (4), 114-117.

Pathak, P., Bhardwaj, N. K., and Singh, A. K. (2011). "Optimization of chemical and enzymatic deinking of photocopier wastepaper," *BioResources* 6(1), 447-463.

Pelletier, A., Li, K. C., Zhao, Y., Court, G., Luo, J., and Frith, M. (2013). "Improvement of enzyme transport in wood chips for thermomechanical pulp refining," *Carbohydrate Polymers* 95(1), 25-31. DOI: 10.1016/j.carbpol.2013.02.025

PN-EN 25264-3 (1999). "Fibrous pulps – laboratory beating – the Jokro mill method," Polish Committee for Standardization, Warsaw, Poland.

PN-EN ISO 5267-1 (2002). "Pulps – Determination of drainability – Part 1: Schopper-Riegler method," Polish Committee for Standardization, Warsaw, Poland.

PN-EN ISO 5269-2 (2007). "Pulps – preparation of laboratory sheets for physical testing – Part 2: Rapid-Kothen method," Polish Committee for Standardization, Warsaw, Poland.

Pulp Paper Mill (2015). "Tissue paper" (http://www.pulppapermill.com/tissue-paper/), Accessed January 17, 2020.

Ramezani, O., and Nazhad, M. M. (2004). "The effect of coarseness on paper formation," *African Pulp and Paper Week* 1-5.

RISI (2016). "Tissue production is increasing demand for market pulp worldwide," (https://www.risiinfo.com/press-release/tissue-production-increasing-demand-market-pulp-worldwide/), Accessed January 10, 2020.

Sain, M., Fortier, D., and Lampron, E. (2002). "Chemi-refiner mechanical pulping of flax shives: Refining energy and fiber properties," *Bioresource Technology* 81(3), 193-200. DOI: 10.1016/S0960-8524(01)00143-2

Salgueiro, A. M., Evtuguin, D. V., Saraiva, J. A., and Almeida, F. (2016). "High pressure-promoted xylanase treatment to enhance papermaking properties of recycled pulp," *Applied Microbiology and Biotechnology* 100, 9885-9893. DOI: 10.1007/s00253-016-7703-5

Saxena, A., and Chauhan, P. S. (2017). "Role of various enzymes for deinking paper: A review," *Critical Reviews in Biotechnology* 37(5), 598-612. DOI: 10.1080/07388551.2016.1207594

Seo, Y. B., Shin, Y. C., and Jeon, Y. (2000). "Enzymatic and mechanical treatment of chemical pulp," *TAPPI Journal* 83(11), 64-73.

Sigoillot, J. C., Petit-Conil, M., Herpoël, I., Joseleau, J. P., Ruel, K., Kurek, B., de Choudens, C., and Aster, M. (2001). "Energy saving with fungal enzymatic treatment of industrial poplar alkaline peroxide pulps," *Enzyme and Microbial Technology* 29(2-3), 160-165. DOI: 10.1016/S0141-0229(01)00368-4

Singh, R., Kumar, M., Mittal, A., and Mehta P. K. (2016). "Microbial enzymes: Industrial progress in 21st century," *3 Biotech*, 6(2), 1-15. DOI: 10.1007/s13205-016-0485-8

Spiridon, I., Duarte, A. P., and Belgacem, M. N. (2001). "Enzymatic hydrolysis of *Pinus pinaster* kraft pulp," *Appita Journal* 54(5), 457-459.

Taipale, T., Österberg, M., Nykänen, A., Ruokolainen, J., and Laine, J. (2010). "Effect of microfibrillated cellulose and fines on the drainage of kraft pulp suspension and paper strength," *Cellulose* 17(5), 1005-1020. DOI: 10.1007/s10570-010-9431-9

TAPPI T230 om-08. (2008). "Viscosity of pulp (capillary viscometer method)," TAPPI Press, Atlanta, GA.

TAPPI T231 cm-96 (1996). "Zero-span breaking strength of pulp (dry zero-span tensile)," TAPPI Press, Atlanta, GA.

Uutela, E. (2016a). "RISI Global Tissue Outlook," (https://events.risiinfo.com/latin-

american-conference/sites/conference/sites/default/files/presentations/2016/Uutela%20Presentation_0.pdf), Accessed September 9, 2020.

Uutela, E. (2016b). "Eastern European Tissue Markets," (http://www.perinijournal.it/Items/en-US/Articoli/PJL-47/eastern-european-tissue-markets), Accessed January 10, 2020.

Valls, C., Pastor, F. I. J., Roncero, M. B., and Vidal, T. (2019). "Assessing the enzymatic effects of cellulases and LPMO in improving mechanical fibrillation of cotton linters," *Biotechnology for Biofuels* 12(1), 161-174. DOI: 10.1186/s13068-019-1502-z

Wang, Y., De Assis, T., Zambrano, F., Pal, L., Venditti, R., Dasmohapatra, S., Pawlak, J., and Gonzalez, R. (2019). "Relationship between human perception of softness and instrument measurements," *BioResources* 14(1), 780-795. DOI: 10.15376/biores.14.1.780-795

Article submitted: July 17, 2020; Peer review completed: October 17, 2020; Revised version received and accepted: April 22, 2021; Published: April 30, 2021.
DOI: 10.15376/biores.16.2.4221-4236