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BLEACHING PRETREATMENT OF SULFITE PULP WITH COMMERCIAL MACERATION ENZYMES

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Abstract

The aim of our paper was to investigate the efficiency of some commercial products that contain blends of maceration enzymes on the bleaching of pulp obtained from soft wood, birch and polar, using a neutral sulfite semi-chemical pulping (NSSC) process. The enzyme biobleaching is a pretreatment process that releases from pulp the lignin-carbohydrate complexes and the hexenuronic acids and reduces the consumption of chemical bleaching agent. The biobleaching efficiency was evaluated by the reduction in the kappa number. The results demonstrate that the efficiency of the maceration enzymes depends on the degree of delignification and on the composition of the blends. The enzymes that contain a blend of pectinolytic enzymes, including polygalacturonase, have significant activity also on mechanically refined pulp. The maceration enzymes that include hemicellulases are highly actives on pulp resulted from chemical extraction and are less active on pulp that was further delignified by mechanical refining. The presence of pectin lyase tends to increase the kappa number due to the release of hexenuronic acids from pectin.

Key words: *biobleaching, sulfite pulp, maceration enzymes, pectinases.*

INTRODUCTION

The utilization of enzymes for pulp bleaching started in the last decades of the 20th century. This biotechnological process was a result of the significant concerns related to the toxic compounds formed during pulp bleaching with chlorine/chlorine derivatives - the adsorbable organic halogens, AOX (Crooks & Sikes 1990), e.g., polychlorinated compounds, with high environment impact due to their bio-accumulation potential (Freire et al., 2003; Solomon et al., 1996).

The brown-yellowish pulp color was related to the presence of the lignin-carbohydrate complexes precipitated on pulp fibers, including the xylan-lignin-glucomannan complex and glucomannan-lignin complex (Kantelinen et al., 1993; Lawoko et al., 2006). Therefore, the main enzymes initially used for enzymatic bleaching were hemicellulases/ xylanases (Viikari et al., 1994). Hemicellulases/xylanases increase the brightness of the pulp due to the solubilization of lignin-carbohydrate complexes and hydrolysis of xylan and mannan. Hemicellulose hydrolysis promotes pulp fibrillation and lamination and cellulose fibers accessibility to bleaching agents (Roncero et al., 2005).

In the last decades, it was demonstrated that the unsaturated 4-deoxy-*β*-l-*threo*-hex-4-enopyranosyluronic acid groups (hexenuronic acids - HexA), formed during the pulping process by partial degradation of the carbohydrate, and HexA complexes with lignin are also involved in the brown-yellowish color of the pulp (Vuorinen et al., 1999). Hexenuronic acids have other adverse effects on pulp bleaching. Due to the presence of unsaturated double bounds HexA determine an increased consumption of chemical bleaching agents / chlorine and leads to an enhanced AOX formation (Nie et al., 2015). Hexenuronic acids are the main compounds responsible for thermal and aging induced yellowing of the bleached pulp (Kuwabara et al., 2012; Sevastyanova et al., 2006). Endo-xylanases remove hexenuronic acids (Shatalov & Pereira, 2009). Laccase activity is complementary to xylanase in removing hexenuronic acids and lignin (Valls & Roncero, 2009). An enzymatic preparation containing lipase A, obtained from *Aspergillus niger* was also proved to be effective in the degradation of the hexenuronic acid (Nguyen et al., 2008). Utilizing the enzymatic cocktails containing, besides xylanase, other enzymes, reduced the bleaching agent consumption and promoted lignin release from the pulp fibers (Immerzeel & Fiskari, 2023).

Recently, it was demonstrated that a combination between xylanases and pectinases has a synergic effect on pulp bleaching, mainly due to the hydrolytic activity of poly-galacturonase on hexenuronic acids (Agrawal et al., 2023; Nagpal et al., 2020; Nagpal et al., 2021). This leads us to the idea to evaluate the effect of commercial maceration enzymes on the bleaching of sulfite pulp.

Commercial maceration enzymes are used in winemaking and fruit juice industry, to improve juice color and aroma extraction (Danalache et al., 2018; Espejo, 2021). These enzyme mixtures includes several classes of hydrolase, such as hemicellulases and pectinolytic enzymes (Toushik et al., 2017; Uzuner & Cekmecelioglu, 2019). As we mentioned already, these hydrolases proved to be effective for pulp (pretreatment) enzymatic bleaching. Therefore, this paper aims to evaluate the effects of several commercial products containing macerating enzyme cocktails on the bleachability of sulfite pulp and on the mechanical resistance of paper resulted from the enzymatically treated pulp.

MATERIALS AND METHODS

Sulfite pulp

The used pulp samples were supplied by Cellulose and Paper Factory (CCH), Drobeta-Turnu Severin (Mehedinți, Romania), and was obtained from softwood, birch and poplar, by a neutral sulfite semi-chemical pulping (NSSC) process.

Commercial enzymes preparations

The following commercial macerating enzyme preparations were used: Safizyme Clean® (Fermentis, Marcq-en-Baroeul, France); VinoTaste® Pro (Novozyme, Bagsværd, Denmark), Zymovarietal Pro Ice (Sodinal, Bucharest, Romania); Enovin Pectinase (Agrovin, Alcázar de San Juan, Spain). Safizyme Clean® is a blend of pectinases, endo polygalacturonase, pectin methyl esterase and pectin lyases. VinoTaste® Pro includes, besides pectinases, β-glucanases, and hemicellulase / arabinanase. Zymovarietal Pro Ice is a mixture of pectinases, including pectin lyase, and β-glucuronidase and it targets especially the aroma and color extraction from grape. Enovin Pectinase is a mixture of pectinolytic enzymes.

Chemicals

The following chemical reagents, analytic purity, were purchased from Sigma-Aldrich (Merck Group, Darmstadt, Germany): Potassium permanganate $KMnO_4$ (used as solution 0.1 N); sodium thiosulfate, $Na_2S_2O_3 \cdot 5\ H_2O$ (used as solution 0.2 N); potassium iodide (KI, used as solution 1 N); sulfuric acid, H_2SO_4 (used as solution 4 N) and Starch (ACS reagent, used as solution 1%). Pure water was produced by reverse osmosis in a Centra 120 system (Elga Veolia Labwater, Celle, Germany).

Kappa number determination

Kappa number was determined in accordance with ISO 302:2015 standard. Kappa number is a measure of pulp bleachability and estimates the quantities of carbon oxidable bonds (COB) by titration with potassium permanganate, according to the following reactions:

$COB + MnO_4^- + H^+ \rightarrow COB_{ox} + H^+ + MnO_4^-$ (*excess*) (*1*)

$2MnO_4^- + 10I^- + 16H^+ \rightarrow 2Mn^{2+} + 5I_2 + 8H_2O$ (*2*)

$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$ (*3*)

Enzymatic treatments

The pulp samples (30 g) were dispersed in 270 mL of pure water and mixed for 5 min, followed by pH adjustment to 7. An equivalent of 5 units per ml pectinolytic activity was added from each commercial enzymes separately. The pectinolytic activity of each enzyme was determined by using dinitrosalicilic (DNS) reagent for reducing sugars (Miller 1959) and poligalacturonic acid as substrate, at 40°C and pH 6.5 (Joshi et al., 2013). One unit is the quantity of enzymes that releases the equivalent of 1 μM reducing sugar per min at 40°C and pH 6.5. The treatment was done for 4 hours at 40°C and pH 6.5. The bio-bleaching efficiency was determined by measuring the reduction in kappa number. Each experiment was repeated three times.

Determination of mechanical characteristics

The tensile strength has been evaluated by the constant elongation gradient method, according to TAPPI T 494 standard, by using a ZwickyLine Z05 dynamometer (ZwickRoell, Ulm, Germany). The double bending resistance has been evaluated according to ISO 5626 TAPPI T423s standard, by using a Schopper type device (IDM Test, San Sebastian, Spain). The assay were done in paper board made on labor according to ISO 5264-2:2011 standard. The assay was done for the mechanical refined NSSC pulp, RAF3.

Statistical analysis

The results from the experiments were submitted to analysis of variance (ANOVA), using the SPSS 21 software package (IBM, Armonk, NY, USA). The statistically relevant differences were established by Fisher's Least Significant Difference (LSD) test.

RESULTS AND DISCUSSIONS

The aspect of semi-chemical pulp resulted from softwood, birch and polar, by using neutral sulfite semi-chemical pulping (NSSC) process, on Cellulose and Paper Factory (CCH), Drobeta-Turnu Severin (Mehedinți, Romania) is presented in Figure 1.

CW **RAF1** **RAF3**

Figure 1. Aspect of semi-chemical pulp resulted from softwood, birch and polar, by using neutral sulfite semi-chemical pulping (NSSC) process, at Cellulose and Paper Factory (CCH), Drobeta-Turnu Severin (Mehedinți, Romania). Left, the initial washed pulp obtained after the chemical treatment of softwood (CW). Middle, the pulp after delignification, the 1st mechanical refining step (RAF1). Right, after delignification, the 3rd mechanical refining step (RAF3)

The initial semi-chemical pulp (CW) is obtained after delignification of soft wood, birch and poplar. The wood chips are boiled with sulfite liquor at 170°C for 2 hours. The preparation of the sulfite liquor involves the following operations: preparation of sodium carbonate solution; melting and burning sulfur; absorption of sulfur dioxide in sodium carbonate solution. The spent (red) sulfite liquor is extracted, and the semi-chemical pulp is washed, resulting the washed chemical pulp - CW.

RAF 1 and RAF 3 are semi-chemical pulp samples, recovered from different mechanical refining steps, from the 1st and, respectively, from the 3rd step.

The mechanical refining step releases lignin-carbohydrate complexes from the pulp by application of the friction and shear forces generated by the pulp milling between steel plates / discs. Such mechanical refining process usually enhances the accessibility of enzymes, due to fibrillation and lamination of the pulp fibers (de Assis et al., 2018).

The initial Kappa number and the lignin equivalent for each sample, from different stages of the NSSC process are presented in Table 1.

Table 1. Initial Kappa number and the lignin equivalent for samples from different stage of the NSSC process

Specification	CW[1]	RAF1[2]	RAF3[3]
Kappa number	103.11±8.23	82.72±5.64	59.06±3.86
Lignin equivalent	15.46±1.24	12.40±0.86	8.85±0.58

[1]CW - the initial pulp obtained after chemical treatment of softwood
[2]RAF1 - after delignification, the 1st mechanical refining step.
[3]RAF3 - after delignification, the 3rd mechanical refining step

The NSSC process retains a large amount of lignin in the pulp. The pulp is further delignified by using refiner mechanical pulping process (Sandberg et al., 2020), in several steps. Sample from the 1st mechanical pulping stage has lower lignin content compared to initial sample, taken from chemical pulping process, and has a higher lignin content compared to 3rd mechanical pulping stage.

The efficacy of the enzymatic treatment on biobleaching the pulp sample from NSSC process is represented in Figure 2. The applied pectinolytic activity was similar. However, the difference comes from the additional enzymes existing in the process. Safizyme Clean® is a blend of pectinolytic enzymes and is highly efficient on the reduction of the kappa number including on the more mechanical refined pulp. Most probably the pectinolytic system, and especially polygalacturonase, is acting on the hexenuronic acids, that are similar with the natural uronic acid substrate (Kumar 2021). Vino Taste® Pro, that includes xylanase, is more effective on the less delignified pulp – that contains more lignin-carbohydrate complexes, which are substrates for hemicellulase/xylanase (Lawoko et al., 2006). Enovin Pectinase and especially Zymovarietal Pro Ice, that contain also pectin lyase, an enzyme from the PL1 family polysaccharide lyases (PL1) class, which produce hexenuronic acids by the degradation of pectin (Lombard et al., 2010), are significantly less effective. On the highly refined mechanical pulp the use of enzyme maceration blends that contain pectin lyase tend to increase the kappa number, most probably due to the release of the hexenuronic acids from the pectin that is still included in the pulp.

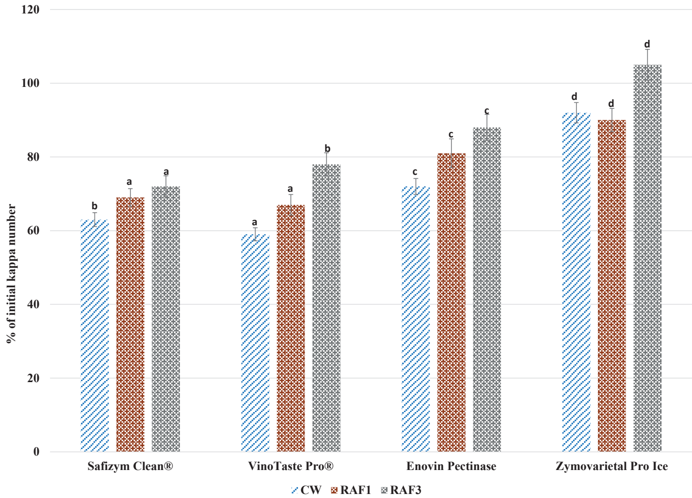

Figure 2. The biobleaching efficacy of treatment with commercial products containing different blends of maceration enzymes on the pulp sample from NSSC process, expressed as percentage of the initial kappa number. CW, the initial pulp obtained after chemical treatment of softwood. RAF1 - after delignification, the 1st mechanical refining step. RAF3 - after delignification, the 3rd mechanical refining step. The values with the same letter do not differ significantly at $p < 0.05$

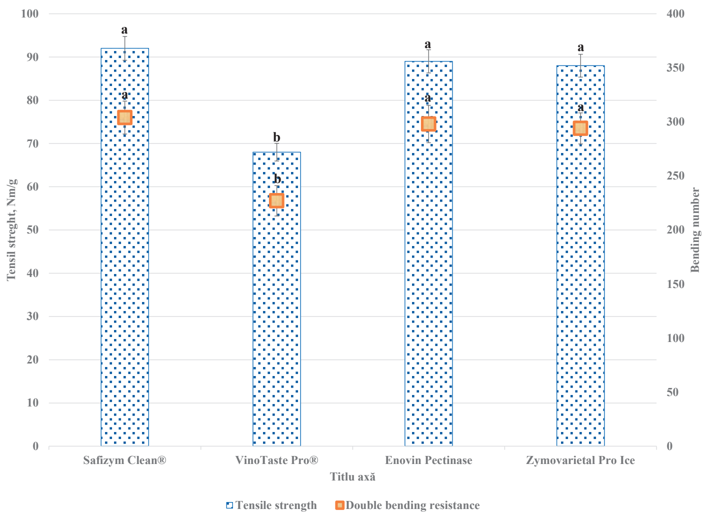

Figure 3. The effects of biobleaching with commercial products containing different blends of maceration enzymes on mechanical properties of the pulps sample from NSSC process, after delignification, the 3rd mechanical refining step (RAF3). The values with the same letter do not differ significantly at $p < 0.05$

In general, the enzymatic treatment does not significantly influence the mechanical characteristics of the pulp - Figure 3. However, VinoTaste® Pro, due to its β-glucanase activity, reduced the tensile strength of the paper produced from the pulp.
Our investigations demonstrate that it is possible to use commercial maceration enzymes for biobleaching of pulp resulted from the NSSC process. The decrease of the kappa number is comparable to that reported for a crude enzymatic preparation containing a xylanase, obtained from *Aureobasidium pullulans* (Christov & Prior, 1994).
The blend of the commercial maceration enzymes has been optimized to get the best results for their application in winemaking and juice production. Our results suggest that it is possible to introduce such blends of commercial maceration enzymes in the composition of the enzymatic cocktails used for the NSSC pulp bleaching. However, it is important to calibrate the enzymatic blends, to maximize bleaching effects and to reduce the negative effects. Better understanding of the effect of pectinolytic enzymes on hexenuronic acids will support a scientific design of the enzymatic cocktails used for pulp biobleaching.

CONCLUSIONS

Commercial products that include maceration enzymes, initially intended to be used in winemaking and juice production, are active also on pulp biobleaching. Most probably the polygalacturonases from the pectinolytic system hydrolyze the hexenuronic acids, releasing them from pulp.
The associated enzymes in commercial maceration blends, such as hemicellulases further support the biobleaching activity, releasing the lignin- carbohydrate complexes from pulp, especially from pulp that it is not mechanically refined. The blends that include polygalacturonase are effective also on the mechanically refined pulp. The enzymatic cocktail that includes pectin lyase tends to increase the kappa number due to the release of hexenuronic acid from pectin. The presence of β-glucanase reduces the mechanical strength of the treated pulp.

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