BEHAVIOUR OF TWO MAIN PORTUGUESE WOOD SPECIES TOWARDS ENZYMATIC HYDROLYSIS

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This paper presents the influence of cellulolytic and hemicellulolytic enzymes on the papermaking properties of pulps from *Eucalyptus globulus* and *Pinus pinaster* wood species from Portugal. It was shown that these enzymes increased the delignification efficiency and degraded the cellulose macromolecules, having effects on the fiber strength. Thus, it was found that these treatments decreased strongly the burst index, but increased significantly the tearing properties. The zero span tensile strength was also affected, especially for *Eucalyptus globulus* pulps. Finally, these treatments were found to increase the brightness of the treated pulps.

Key words: xylanase, cellulase, *Pinus pinaster, Eucalyptus globulus*, enzymatic treatment, paper properties

INTRODUCTION

At present, the pulp and paper industry in Portugal is using mainly two wood species, namely: (i) *Eucalyptus globulus* and (ii) *Pinus pinaster*. The first one is a hardwood used in the production of bleached kraft pulps which are used essentially for photocopy and written papers. The second species is a softwood used to obtain unbleached kraft pulp for packaging papers. Kraft pulps from *Pinus pinaster* are generally darker than those obtained from other common softwoods (e.g. *Pinus sylvestris*). Moreover, they were found to have a bad bleachability. Thus, Portuguese pulp and paper industry imports bleached softwood long fibres from Northern countries.

Recently, our department has been involved in a large program aiming at getting deep knowledge about the origin of pulp coloration in order to improve the quality of unbleached kraft pulps from *Pinus pinaster*.[1-5] These studies were oriented into two directions: (i) establishing of the lignin structure and identification of chromophore groups which might be responsible for coulouring the pulps from Portuguese pine and (ii) the role which the degradation products of extractives could play in colouring the pine pulps.

The application of biotechnological processes to improve pulp and paper manufacturing processes and products is gaining global attention. In fact, application of biotechnologies in the pulp and paper industry is now a reality.[6] Enzymes are being produced on a commercial scale and are available at a relatively low price. Research to establish the effect of enzymatic treatment continues in an effort to improve the effect of enzyme bleaching. At the same time, xylanase systems have been developed to ensure selective hydrolysis of the xylan hemicellulose without cellulose attack. Hemicellulolytic enzymes have been found to be commercially feasible for pulp bleaching.[7] Thus, xylanase treatments facilitate chemical extraction of lignin from pulp, with important reduction of bleaching chemical consumption and beneficial effect on the environment. Enzymes are widely used as a pretreatment stage to improve the bleaching capacity of pulp.[8-12]

The present paper is an additional contribution on this field, studying the potential usage of hydrolytic enzymes to enhance the fiber characteristics of pulps derived from these species.

EXPERIMENTAL

Material

Eucaliptus globolus pulps (with Kappa number 17.5) and *Pinus pinaster* wood chips were a kind gift from Raiz and Portugal Tejo Company, respectively. The pine chips (30 x 10 x 3 mm) were cooked using conventional kraft conditions: alkali active 21%; sulfidity 30 %; liquid to wood ratio 5:1; 90 minutes of heating from room temperature to 170^0 and maintaining the reactor at this temperature for 90 min and 120 min, respectively. The Kappa number of pine pulp was 46.2.

Enzymatic treatment

Enzymatic treatment was performed at a temperature of 50^0, using a mixture of cellulase named Celluclast. Two cellulase and hemicellulase mixtures, Cartazyme HS and Novozym, were also used, at doses presented in Table 1. The pulp consistency was 10 % and treatment time - 120 minutes.

TABLE 1

Conditions of enzymatic treatment

Enzymatic preparation	Cellulase, UI / g od	Xylanase, UI / g od
Cartazyme HS	0.001	0.05
Novozym	0.05	0.007
Celluclast	0.5	-

Pulp analysis

Kappa number and viscosity were determined according to Tappi methods: T 236 cm-85 and T 230-om 89, respectively. The sheets were obtained using a Rapid Kothen apparatus (T 205 om-88) and the papermaking properties were evaluated by Tappi methods, as follows: T 403 om-91 - burst, T 404 cm-92 - tensile and T 496 cm-85 - tear resistance. The beating degree was determined according to SCAN C19-65 standard; the fiber classification was realised using a Bauer MacNett apparatus, using the Tappi standard T 233 cm-82. Brightness was measured with a Technidyne ISO 2 colorimeter.

RESULTS AND DISCUSSION

The effects of enzymatic treatments on pulp delignification and viscosity are presented in Table 2.

TABLE 2

Influence of the enzymatic treatment on the Kappa number and viscosity of the treated pulps

	Eucalypt				Pine			
	Reference	Carthazyme	Celluclast	Novozym	Reference	Carthazyme	Celluclast	Novozym
Kappa number	17.52	15.89	14.83	15.83	46.21	44.96	42.57	44.55
Viscosity, mPa.s	33.154	31.682	17.853	20.713	31.552	31.124	18.689	22.121

For both pulps, all enzymes used were found to improve the delignification level, but the decrease in Kappa number after xylanase treatment was much less for softwood compared to hardwood pulps. Indeed, for *Eucalyptus globulus* pulp, the mixtures of hemicellulase and cellulase (Carthazyme and Novozym) have induced a decrease of 1.7 units of Kappa number while, for cellulase, a decrease of 2.3 units has been obtained. For pine pulp, the use of cellulase-hemicellulase mixtures led to a decrease of 2 units of Kappa number (compared with the reference), whereas cellulase produced a more pronounced effect on the delignified pulp (Kappa number decrease by about 3.5 units).

The fibre classification was also carried out and results obtained being presented in Figures 1 and 2, for *Eucalyptus globulus* and *Pinus pinaster* pulps, respectively. A first general comment is that there are significant changes in the fiber fraction, as a function of the enzymatic preparation used.

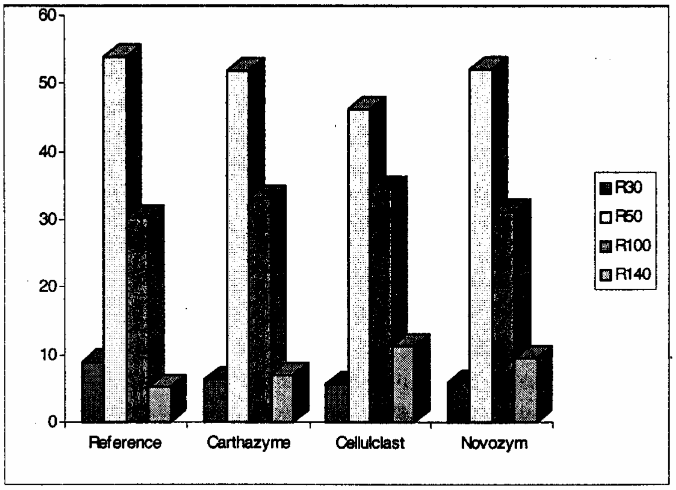

Fig. 1 – Influence of the enzymatic treatment on the eucalypt fiber length.

Thus, for *Eucaliptus globulus* pulp (in which fraction R50 constitutes the major part), the cellulase-hemicellulase mixtures influenced both the R50 and R100 fractions, while cellulase alone affects especially the R50 fraction.

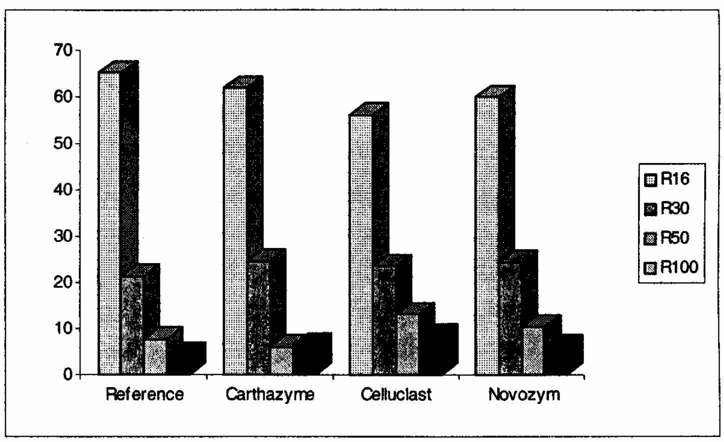

Fig. 2 – Influence of the enzymatic treatment on the pine fiber length.

For *Pinus pinaster* pulp, composed mostly of long fibers (R16 and R30), the screening results show that these fibers are slightly affected by cellulase-hemicellulase mixtures, while cellulase alone affects them more intensely. This demonstrates the protective effect of cellulase-hemicellulase mixtures on cellulosic fiber, as confirmed by the evolution of viscosity (see Table 1), evidencing the destructive effect of Celluclast on cellulose macromolecular chains. This could be attributed to the action of the endoglucanase component, that induces disintegration of fiber with increasing the fines content.

For all enzymatically treated pulps, the fines content increases, especially for cellulase treatment. The combined action of xylanase and cellulase does not intensify the fiber disintegration, while the enzymatic treatments do not appear to adversely affect the beating degree and physical properties of pulps. Thus, pulp freeness for enzyme-treated pulps was higher than that of the untreated ones, as seen in Figure 3. This increase in the beating degree was probably due to fiber defibrillation and preferential hydrolysis of fines.

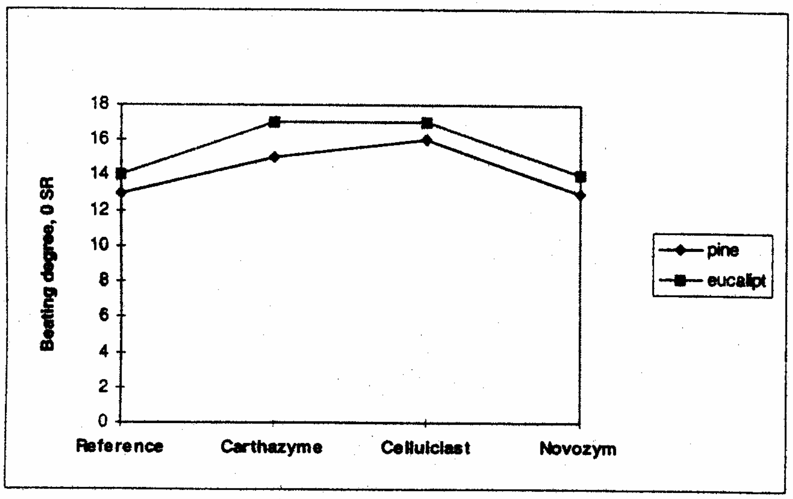

Fig. 3 – Influence of the enzymatic treatment on the beating degree.

Tensile strength is a direct indication of the durability and potential end-use performance of paper, which is generally submitted to tensile stresses during its use. The breaking length of the sheets, presented in Figure 4, is influenced by both the fiber length and variations in the level of bonding. It is evident that shorter fiber fractions produce denser handsheets and have a higher tensile index, which indicates a tighter fibrous network with a closer fiber-to-fiber contact.

The burst index decreases for all enzyme-treated pulps, probably due to fiber fibrillation and loosening of intrinsic fiber strength.

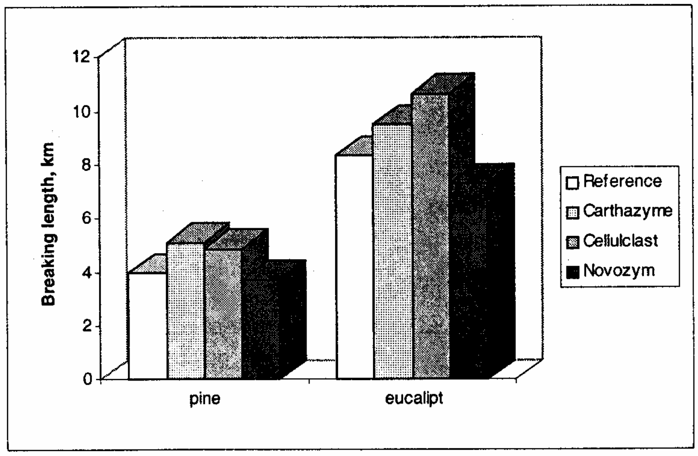

Fig. 4 – Influence of the enzymatic treatment on the breaking length.

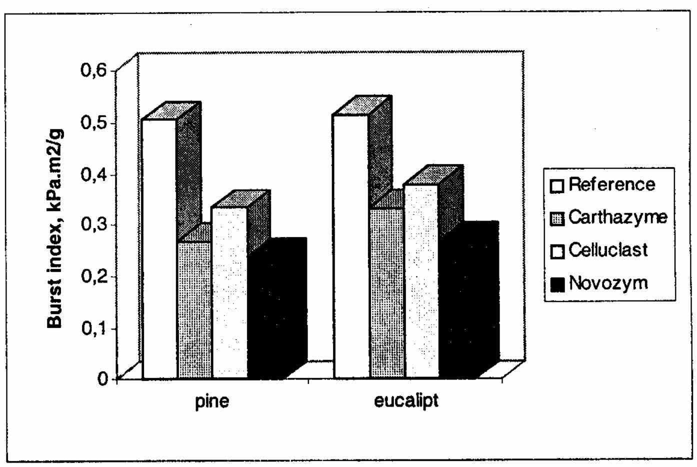

Fig. 5 – Burst index of enzymatically treated pulps.

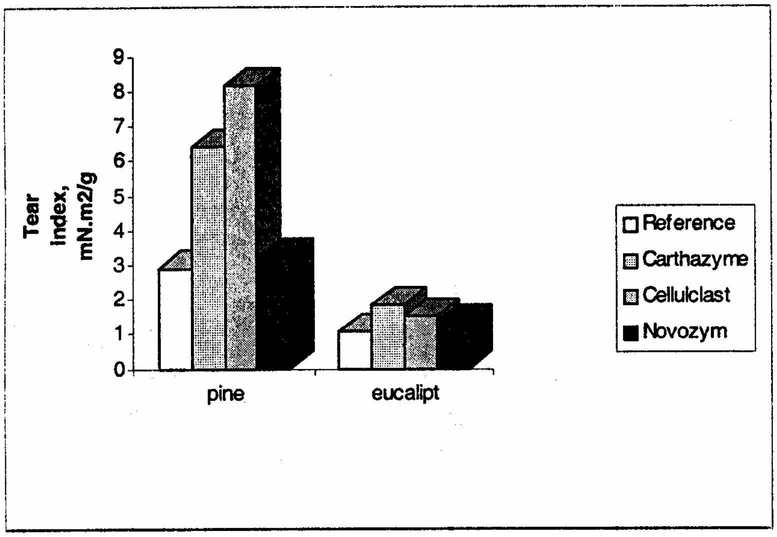

Fig. 6 – Tear index evolution for pulps after enzymatic treatments.

Tearing strength is a widely used test in the evaluation of paper's mechanical properties. Page[13] demonstrated the relative importance of fiber length, fiber strength and coarseness to tear resistance. Thus, in poorly-bonding sheets, tearing resistance depends more on fiber length, while the opposite was recorded for a well-bonded sheet, which explains the important increase of tearing index obtained for the pine pulp (constituted especially of long fibers, the enzymatic treatment determining an increase of the R 30 and R 50 fractions). As seen in Figure 6, for *Eucalyptus globulus* pulps (with a few long fiber content, where the most affected was the R30 fraction) only a slight improvement for this index was observed.

The wet-zero span test indicates the influence of fiber bonding on the zero-span tensile strength. This is based on the assumption that, in wet sheets, all interfiber bonds are effectively broken. The wet-zero span tensile strength is lower for a wide range of pulps, due to weakening of the individual fibers which make up the paper sheets. This loss in strength depends on the extent of chemical and mechanical damages of the interfibrillar matrix of the fibers during processing.[14] Weakening of the lignin-hemicellulose matrix holding the cellulose microfibrils together determine the reduction of zero-span strength upon wetting. Treatments with hemicellulases and cellulases could degrade the interfibrillar matrix. Zero-span tensile decreases by the reduction of fiber length and increases by a higher level of bonding in the sheets.[15] That is why, for all enzymatically-treated pulps, a reduction of the wet-zero span tensile strength was noticed, as seen in Figure 7.

The most important reduction was obtained for Novozym treatment, for both pulps, which is probably due to the high content of cellulases.

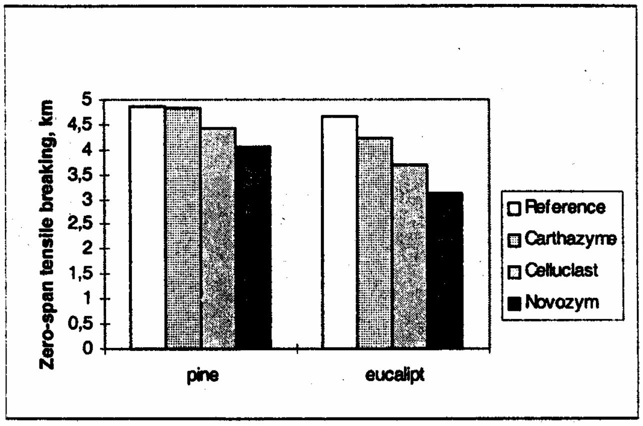

Fig. 7 – Influence of the enzymatic treatment on the zero-span tensile breaking.

Finally, the influence of the enzymatic treatment on the brightness of pulps was carried out. It was found out that the enzyme treatment of pulp with xylanase improved pulp brightness, as seen in Figure 8. Carthazyme preparation was found to be more effective in increasing brightness, in comparison with other enzyme mixtures. A gain of 1.6 points for pine pulps and 6 points for eucalypt pulp was reached by the application of this treatment.

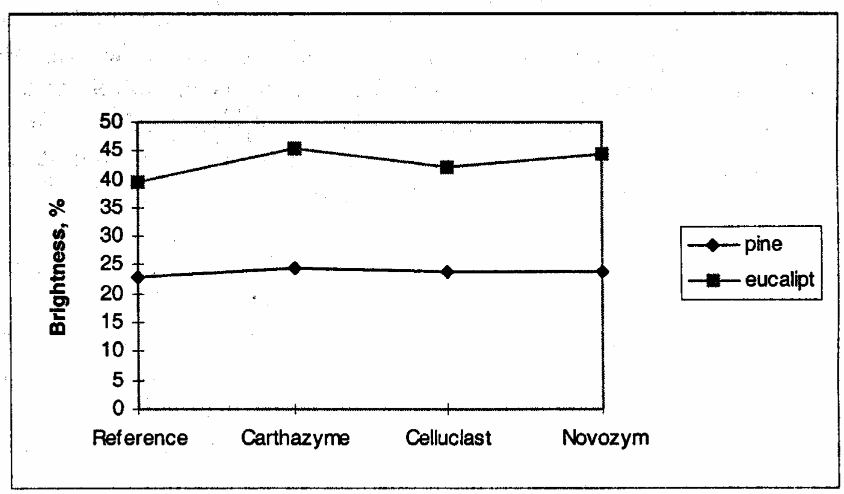

Fig. 8 – Influence of the enzymatic treatment on brightness.

The increase of brightness was accompanied by a decrease in the Kappa number, indicating the removal of a part of residual lignin in pulp. It is evident that xylanase preparations are particularly effective in facilitating bleaching of hardwood pulp. However, the effect on softwood pulp is less pronounced, which may be due to the high xylan content of hardwood pulp compared with the softwood one.

CONCLUSIONS

The overall results with hemicellulases were positive and suggest that implementation of enzymatic treatment before bleaching seems to be promising in reducing chemicals' bleaching consumption and environment pollution.

These conclusions led to a new set of experiments, aiming at verifying whether the enzymatic pretreatment would demonstrate some practical benefits in Portuguese pulp and paper industry, or not.

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