IMPROVEMENT OF TCF BLEACHING OF OLIVE TREE PRUNING RESIDUE PULP BY ADDITION OF A LACCASE AND/OR XYLANASE PRE-TREATMENT

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This study aimed at assessing the biobleachability of soda pulps obtained from olive tree pruning residue. The enzymatic (LMS) pre-treatment was applied prior to a simple totally chlorine free (TCF) bleaching sequence, consisting of an alkaline extraction and a hydrogen peroxide stage. Additionally, the effect of adding xylanase jointly with or prior to LMS was evaluated. All of these enzymatic pre-treatments were associated with an enhancement of the bleaching sequence. The best results were found when both enzymes were applied in the same stage: lowest hydrogen peroxide consumption (63 percent); kappa number, 11.6; brightness, 46 percent ISO. The mechanical properties observed were similar to those reported by other authors who have studied pulps from olive tree pruning residue. Finally, bleached pulps were subjected to accelerated ageing in order to assess the evolution of brightness and colorimetric properties. Although biobleached pulps showed lower stability upon ageing, the best optical properties, even after ageing, were observed in pulps treated with both xylanase and laccase.

Keywords: Olive tree pruning residue; Alternative raw materials; Xylanases; Laccases; Biobleaching.

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INTRODUCTION

The 2009 worldwide production of paper and cardboard was 376.8 millions of tons, which meant a 6.4 percent increase in production from 2004 (FAO 2011). In Spain, about 6 millions of tons of paper and cardboard were produced in 2010, and that same year consumption per capita was approximately 160 kg (ASPAPEL 2011). These figures highlight the need for using and applying increasingly clean technologies in these processes, where saving energy and water go hand in hand with more efficient use of raw materials, so that the goal of sustainable development is finally within reach.

It is on the basis of this principle that the idea of abandoning conventional raw materials in favor of alternative options is gaining momentum. These alternative raw materials comprise non-woody plants and residues from agriculture and forestry such as olive tree pruning, cereal straw, sunflower stalks, or vine shoots. The advantages of using these raw materials include their availability in certain geographic regions where water is scarce and/or forest resources are limited, the added value of using agri-food residues, and the potential to produce "special" papers by virtue of the wide variability in

morphology and chemical composition of all these alternative raw materials. In spite of that, they are not free from disadvantages such as a very short harvest period or their high ash and extractive contents, which contribute to a low pulping yield.

The olive tree (*Olea europaea*) is a typical Mediterranean plant that is extremely well adapted to the climatic features of the region in which it is indigenous: mild, rainy winters, and hot, dry summers. According to data published by FAO, in 2009, olive plantations in Spain covered approximately 2,500,000 hectares, generating 53 percent of the European Union's production that same year, and making Spain the biggest producer in the world, followed by Italy (25%) and Greece (14%). Twice a year, olive trees are pruned for regeneration, creating a large amount of waste, which is a major problem for the agricultural industry. The present solution is collecting and shredding the prunings to subsequently spread the resulting material back onto the fields, which turns out to be an unwise practice due to its cost and the proliferation of pests. Another common solution to the problem is to burn these residues; in theory, this is a controlled practice, but often it is a cause of man-provoked fires. An alternative solution supported by several studies is processing lignocellulosic agricultural residues to obtain cellulose fibers that are destined to the pulp and paper industry.

Several research efforts have focused on the production of paper pulp from olive tree prunings. Kraft, soda, and organosolv pulping processes have been studied to this end (Jiménez et al. 1999, 2001, 2008; López et al. 2001). Pulping processes such as Alcell (ethanol and water), MD Organocell (ethanol and soda), or Organocell (methanol and soda anthraquinone) have also been widely used (Jiménez et al. 2004). López et al. (2000) have compared kraft, soda, and sulphite pulping of olive tree pruning residue and reported that soda pulping is the least polluting and provides high yields and pulps with high holocellulose content (79.7%).

In a different line of research, the use of microbial enzymes to treat pulps before applying the standard bleaching sequences is considered a valuable alternative to be applied in the pulp and paper industry. These enzymes could help to address the environmental concerns and the low selectivity inherent to the application of the Elemental Chlorine Free (ECF) and Totally Chlorine Free (TCF) bleaching sequences, respectively. Many researchers have pretreated pulps with enzymes, mainly with laccase, and have reported that enzymatically pretreated pulps require smaller amounts of chemicals in subsequent bleaching, that the quality of the pulps is maintained, if not improved, and that the pollutant load of the process effluents is reduced (Eugenio et al. 2010; Moldes et al. 2008; Moldes and Vidal 2011; Valls et al. 2012; Viikari et al. 1986). The interest of the use of laccases lies in the fact that these enzymes oxidize a wide variety of phenolic compounds (Thurston 1994) with only the requirement of molecular oxygen. Moreover, the combination of laccases and low molecular weight chemical compounds (laccase-mediator system) can extend the oxidative action of laccases to non-phenolic structures (Bourbonnais and Paice 1992). However, the industrial use of laccase-mediator systems (LMS) still has some drawbacks, such as the toxicity of some mediators, the cost of the enzymes and the optimization of the industrial scalability. Therefore, researchers are still working in this field, trying to optimize the production of enzymes, to find natural mediator with less environmental problems, and to optimize the conditions for industrial operations.

Xylanases have also been applied to biobleaching processes, as their removal of the xylan layer enhances the bleaching effect of chemical reagents (Birijlall et al. 2011; Ko et al. 2011; Valls and Roncero, 2009). Thus, numerous biobleaching studies using xylanases or laccases have been published (Chauhan et al. 2006; Fillat et al. 2010; Eugenio et al. 2011). However, few studies have been found that assess both enzymes in sequence or jointly (Oksanen et al. 1997; Bajpai et al. 2006; Kapoor et al. 2007; Valls and Roncero 2009). Finally, to the best of our knowledge, the application of these enzymes to pulps obtained from olive tree pruning residue has not been studied yet.

Consequently, the main objective of this study was to evaluate the feasibility of using commercial xylanase and laccase, in sequence or jointly, as a previous treatment to a standard TCF bleaching sequence acting on soda anthraquinone pulp obtained from olive tree pruning residue. To this end, delignification degree, viscosity, brightness, CIE L*a*b*, and CIE L* C* color coordinates and mechanical strength were analyzed. Finally, the consumption of hydrogen peroxide was also measured, as well as the optical properties before and after an accelerated ageing process of the resulting paper sheets.

EXPERIMENTAL

Chemicals and Raw Materials

All chemicals were reagent-grade and obtained from Merck (Barcelona, Spain), except for the 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonate (ABTS), which was purchased from Roche (Madrid, Spain). The commercial enzymes used in this study were Novozym 51003 (laccase) and Pulpzyme HC (xylanase), produced by submerged fermentation of genetically modified *Aspergillus* sp. and *Bacillus* sp., respectively. Both enzymes were supplied as donations by Novozymes (Bagsvaerd, Denmark).

Olive tree pruning residue was supplied by "Cooperativa Agrícola San Fº de Asís" (Montefrío, Granada, Spain). The chemical composition of this raw material was: 1.4%, ash content; 6.7%, hot water solubles; 17.4%, 1% NaOH solubles; 8.0% ethanol extractives; 20.7% Klason lignin; 89.2% holocelluloses; and 49.6% α-cellulose.

Pulp Production

Pulping process

Olive tree pruning residue pulp was obtained using a cylindrical 15 L batch reactor heated by electrical wires. The reactor incorporated a rotating axle to ensure proper agitation and was connected to a control unit equipped with appropriate mechanisms for measuring and controlling pressure and temperature. Cooking conditions with soda-anthraquinone in the reactor were as follows: 175ºC, 15% NaOH, 1% AQ for 120 minutes, and an 8 to 1 liquid/solid ratio. Material cooked under these conditions was subsequently washed to remove residual cooking liquor and then fiberized in a wet disintegrator at 1200 rpm for 30 minutes, which was followed by beating in a Sprout-Bauer refiner. Finally, the fiberized material was passed through a screen (mesh size, 0.16 mm) to remove uncooked particles.

Oxygen delignification

Pulp obtained from the soda-anthraquinone process was treated with oxygen for further delignification. This treatment was carried out in a 20-liter rotary reactor with a jacket-type electrical heater controlled by a computer to set and maintain the treatment temperature. Conditions of oxygen delignification were 110ºC, 60 minutes, 0.6 MPa of oxygen pressure, 3% NaOH o.d.p. (over dry pulp), 0.5% MgSO4 o.d.p., and 10% consistency.

Pulp before and after oxygen delignification was characterized by determining their content of ash (T211 om-93), hot water solubles (T207 om-93), 1% NaOH solubles (T2012 om-07), ethanol extractives (T204 cm-07), Klason lignin (T222 om-88), holocellulose (T9 m-54), and α-cellulose (T203 OS-61).

Enzymatic Pre-treatments

The pulp obtained from olive tree pruning residue was subjected to a laccase treatment using a commercial enzyme (L). A commercial xylanase was also used in a previous step or jointly with the laccase treatment (XL or X+L, respectively) with the intent of assessing whether or not it would enhance the laccase effectiveness.

The laccase treatment was conducted in duplicate in 500 mL pressurized reactors, adding the commercial laccase and chemicals to 50-g of pulp from olive tree pruning residue and blending intensively before adding oxygen at a pressure of 6 kg/cm^2. Both reactors were submerged in a thermostatic bath at 45ºC for 2 hours. The pH was kept at a value of 6.5 by using phosphate buffer (100 mM). Temperature and pH were selected as per the product specification sheet. During the laccase treatment, consistency, laccase dose and mediator concentration were fixed at constant values: 10% (w/v), 17.5 U/g over dried pulp (odp), and 0.05 mmol/g odp acetosyringone (4-hydroxy-3,5-dimethoxy-acetophenone), respectively. Also, 2-3 drops of 0.05% (v/v) Tween 80 were added in all assays, in order to improve the interaction between enzyme and substrate. When xylanase was added to test its influence during the laccase treatment, the concentration was 20 AXU/g odp. When a xylanase treatment was carried out as a previous step to the laccase treatment, conditions were as follows: 10% (w/v) consistency, 2 hours, 20 AXU/g odp, 45ºC temperature, and pH 6.5 (as per the product specification sheet).

Controls were included in the experimental design as follows: i) EP: all enzymatic treatments omitted (designated as conventional bleaching); ii) LcEP: bleaching carried out in the absence of both laccase and mediator, but in the presence of the rest of ingredients used in the laccase treatment — pH 6.5, 6 kg/cm^2 O_2 and 45°C for 2 hours; iii) XcLcEP: bleaching carried out with two enzymatic treatments without the corresponding enzymes, but with the rest of ingredients used in both enzymatic treatments — pH 6.5, 6 kg/cm^2 O_2 (only in Lc stage) and 45°C for 2 hours. The control (X+L)cEP was not assayed because it is equivalent to the LcEP control.

Alkaline Extraction and Hydrogen Peroxide Treatments

After the enzymatic pre-treatment, pulps were washed with distilled water and air-dried at room temperature. This was followed by an alkaline extraction (E) stage under the following conditions: NaOH, 1.5% odp; consistency, 5%; temperature, 90ºC; and treatment duration, 120 minutes. Thereafter, pulps were washed again, and a hydrogen

peroxide bleaching stage (P) was applied under the following conditions: H_2O_2, 3% odp; NaOH, 1.5% odp; DTPA, 1% odp; $MgSO_4 \cdot 7H_2O$, 0.2% odp; consistency, 5%; temperature, 90ºC; and treatment duration, 90 minutes. Operational conditions used in the alkaline extraction and the hydrogen peroxide treatments were selected based on previous reports (Eugenio et al. 2011; Martin-Sampedro et al. 2012). Residual hydrogen peroxide in the bleaching effluent was determined by standard iodometric titration.

Pulp and Paper Characterization

After each stage of the bleaching sequence, treated pulps were characterized, according to the corresponding standards, in terms of their kappa number (UNE 57034), viscosity (UNE 57-039-92), and brightness (UNE 57062). Handsheets were obtained from the treated pulps in accordance with UNE-EN ISO 5269-2 and characterized in terms of tensile and tear indexes (UNE-EN ISO 5270). CIE L*a*b* and CIE L*C* coordinates (T 527) were also determined in the handsheets using a spectrophotometer ELREPHO 070 (Lorentze and Wettre).

Accelerated Ageing

The bleached and the unbleached handsheets were subjected to accelerated ageing to analyze the evolution of their optical properties. The accelerated ageing was carried out in a climatic test cabinet CTS (model C-20/250/S) as a moist heat treatment at 80ºC and 65% relative humidity that lasted 6 days, as specified by the standard UNE 57092-4. After accelerated ageing, pulps were characterized in terms of brightness and CIE L*a*b* and CIE L*C* color coordinates also in keeping with the aforementioned standards.

RESULTS AND DISCUSSION

After soda-anthraquinone pulping, the olive tree pulp obtained showed a kappa number and viscosity of 38.7 and 794 mL/g respectively, and a brightness of 18.4 % ISO. The chemical composition of this pulp was: 2.3% ash content; 1.4% hot water solubles; 3.0% of 1% NaOH solubles; 4.6% ethanol extractives; 6.3% Klason lignin; 86.9% holocelluloses; and 35.0% α-cellulose. In order to reduce the lignin content of this pulp and improve the effectiveness of the subsequent bleaching process, an oxygen delignification step was carried out before entering the biobleaching sequence. Parameters of the resulting oxygen-delignified pulp were kappa number 19.0; viscosity 680 mL/g; and brightness 29.9% ISO. The chemical composition of this pulp was also determined: 2.0% ash content; 0.5% hot water solubles; 3.1% of 1% NaOH solubles; 4.4% ethanol extractives; 3.5% Klason lignin; 89.0% holocelluloses; and 34.9% α-cellulose.

Afterwards, several biobleaching sequences were carried out, all of them consisting of an enzymatic treatment (with xylanase and/or laccase) followed by a TCF bleaching sequence (alkaline extraction plus hydrogen peroxide bleaching), as described in the experimental section.

Pulp Characterization

Changes in kappa number, viscosity, and brightness of the olive tree pulp, after each stage in the different biobleaching sequences are shown in Figs. 1 to 3, respectively, . The unbleached pulp (oxygen delignified) was used as control.

The kappa number decreased after each stage of all bleached sequences with one enzymatic/control stage, the biggest reduction taking place after the hydrogen peroxide stage (Fig. 1). These results are consistent with those reported elsewhere (Eugenio et al. 2010, 2011; Valls and Roncero 2009). However, with the two enzymatic treatments carried out in sequence, a slight increase in the kappa number was found after the laccase stage. This increase in the kappa number could be associated with grafting of the natural mediator (acetosyringone) to the fibers. It has been suggested that mediators can remain temporarily stuck to the fibers, consuming reagent during the kappa number determination (Dyer and Ragauskas 2004; Cadena et al. 2011; Fillat et al. 2012).

If the sequences are compared, it can be observed that, when enzymes are added (LEP, (X+L)EP and XLEP), the final kappa number is smaller than in controls and in the conventional bleaching sequence EP. A plausible explanation for this finding could be that these enzymes render lignin easier to remove in the subsequent chemical bleaching, as has been reported also elsewhere (Eugenio et al. 2010, 2011; Fillat et al. 2010; Martin-Sampedro et al. 2011a; Valls and Roncero 2009). Similar delignification rates were obtained after the three biobleaching sequences (37.5% LEP, 38.8% (X+L) EP, and 37.3% XLEP), but when xylanase and laccase were applied in the same step, the delignification rate was highest. Other researchers have reported that the xylanase treatment enhances the laccase treatment (Surma-Ślusarska and Leks- Stępień 2001; Valls and Roncero 2009). However, most studies report that, when both enzymes are applied in sequence, there is more delignification than when they are used jointly in a single step (Surma-Ślusarska and Leks- Stępień 2001; Oksanen et al. 1997; Martin-Sampedro et al. 2012). Our results contradict these reports, and perhaps the reason lies in the raw material used, olive tree pruning residue instead of birch, pine, or oil palm empty fruit bunches used in other studies. Nevertheless, it must be pointed out that the difference between the XLEP and (X+L)EP kappa numbers was less than 0.3.

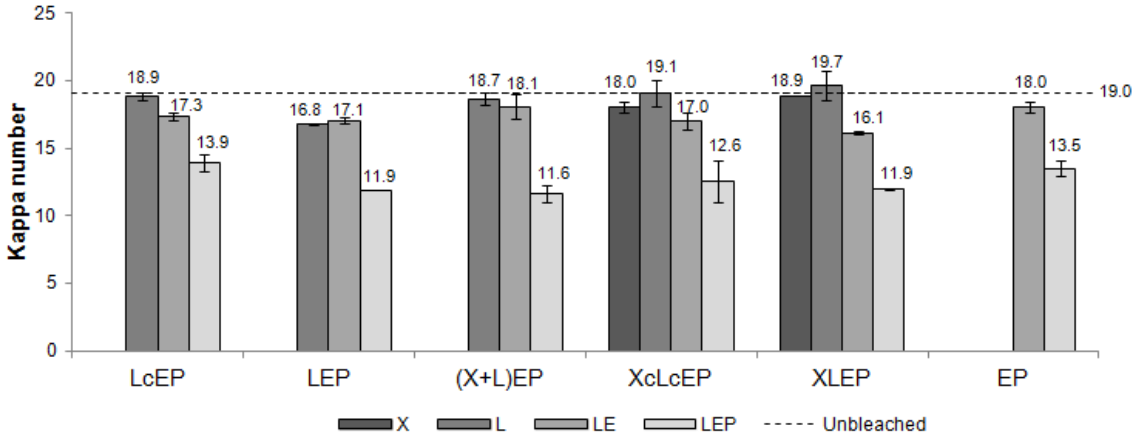

Fig. 1. Kappa number of the unbleached pulp and after each stage of the different biobleaching sequences

Figure 2 shows the evolution of viscosity after each stage of the bleaching sequences. As it is shown, no big differences were found at the end of the sequences, and all bleached pulps presented a viscosity of 650-700 g/mL. According to SCAN-C15, the degree of polymerization (DP) of cellulose can be deduced from the pulp's viscosity. Thus, all bleached pulps had a DP in the range of 930 to 1010. These results indicate that the biobleaching process causes no significant degradation of the polysaccharide chains, which is consistent with findings reported elsewhere (Eugenio et al. 2010; Oudia et al. 2008). However, after the stage in which xylanase and laccase were applied jointly, an increase in viscosity was observed. Similar increases after xylanase treatment have been attributed to the elimination of hemicelluloses of low molar mass due to xylanase action (Gonçalves et al. 2008; Roncero et al. 2005). In our study, when xylanase was applied in a previous step (XLEP), no increase in viscosity was observed after the enzymatic treatment. However, viscosity did increase after the alkaline extraction in this sequence and also in (X+L) EP. These results could indicate that, when xylanase was applied (in sequence or jointly with laccase), some hemicellulose chains of low molecular mass were shortened and converted into alkaline-soluble material that was removed in the subsequent alkaline extraction.

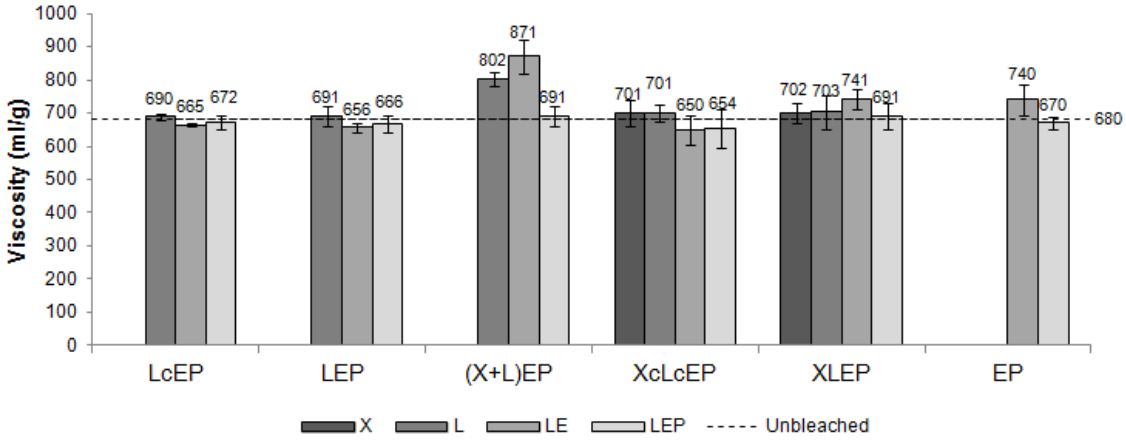

Fig. 2. Viscosity of the unbleached pulp and after each stage of the different biobleaching sequences

Figure 3 shows an increase in brightness in the course of the bleaching sequences, which is consistent with other results reported previously (Eugenio et al. 2010, 2011). As we expected, brightness increased the most during the hydrogen peroxide stage. A decrease in brightness after the laccase treatment, which is associated to the formation of chromophores during the oxidation of lignin by the LMS, has also been reported (Fillat et al. 2010, Martin-Sampedro et al. 2011a, Eugenio et al. 2010), but this was not observed in our present study, and attention must be drawn to the fact that raw materials differ across studies (flax, *Eucalyptus globulus*). Another important difference between ours and other studies is that final brightness was significantly lower in our sequences, also likely a consequence of the raw material that was used. Brightness of pulp from olive tree pruning residue was increased from 30 to 44 percent ISO after the LEP sequence, whereas brightness of *E. globulus* pulp treated with a similar LEP sequence (with the

same commercial laccase), has been reported to increase from 34 to 63 percent ISO (Martin-Sampedro et al. 2011a). Comparable increases in brightness have also been reported after applying fungal or bacterial laccase in an LEP sequence to raw materials such as *E. globulus*, flax, or oil palm empty fruit bunches (Eugenio et al. 2010, 2011; Fillat et al. 2010, Martin-Sampedro et al. 2012). The lower bleachability of the olive tree pruning residue pulp could be due to differences in the lignin structure or the presence of different chromophore groups in the original pulp that make the subsequent bleaching difficult. Similar brightness has been obtained by López et al. (2003), who have reported on the bleaching of kraft pulp from olive tree residue using hydrogen peroxide, ozone, or chlorine dioxide as bleaching agents.

In all cases, enzymes brought about higher increases in brightness compared to the respective control sequences, which is consistent with the results found for the kappa number. Brightness was highest in sequences in which both xylanase and laccase were applied, regardless of whether both enzymes were applied jointly or in sequence. Therefore, it can be concluded that xylanase enhances the laccase treatment, as other authors have reported (Oksanen et al. 1997; Surma-Ślusarska and Leks-Stępień 2001). These researchers suggest that, by eliminating some of the xylan on the fiber surface, the xylanase treatment favors enzyme and/or chemical access, therefore, affording better pulp bleaching.

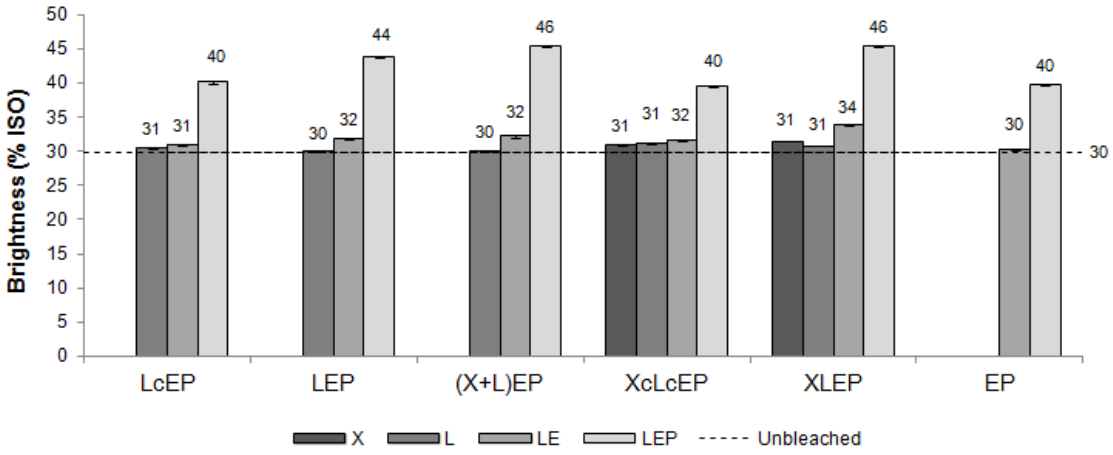

Fig. 3. Brightness of the unbleached pulp and after each stage of the different biobleaching sequences

Besides brightness, the color appearance of paper is also an important parameter in many different applications, and good white base colors are necessary where products are to be dyed, printed, or otherwise colored. Therefore, a numerical definition of color is essential for good quality control and for a good customer–producer relationship. For this reason, CIE L*a*b* and CIE L*C* color coordinates (Fig. 4) were measured in unbleached pulp and, after the P stage, in all bleached pulps. Results were compared in order to evaluate color changes produced during the biobleaching and conventional bleaching processes, since the color appearance of paper is an important parameter in many different applications.

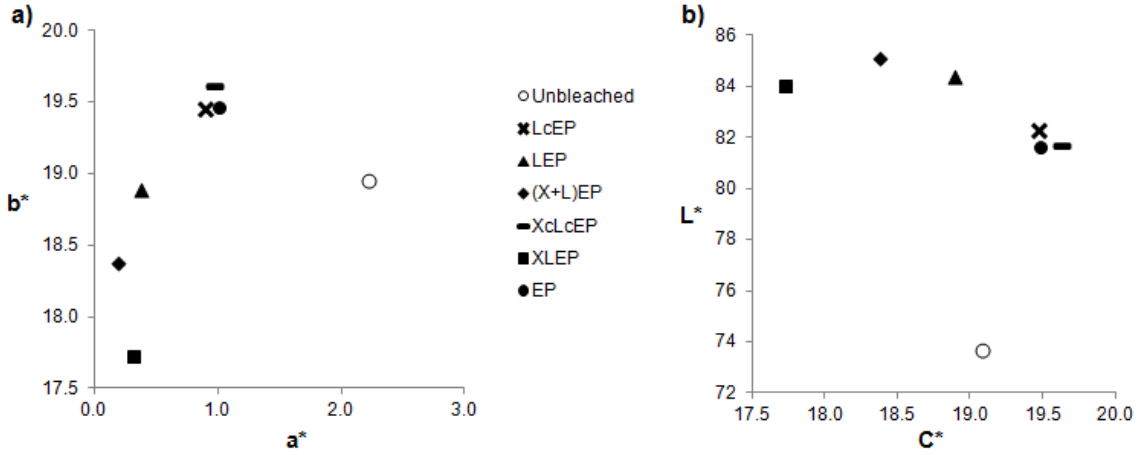

Fig. 4. CIE L*a*b* and CIE L*C* color coordinates of unbleached pulp and bleached pulps at the end of the bleaching sequences

After the biobleaching sequences in which enzymes were added, all pulps increased L* and decreased C* (i.e. lightness increased and color decreased), compared to the unbleached pulp. The same behavior has been observed when applying xylanase and/or LMS to different raw materials (Eugenio et al. 2011; Fillat et al. 2012; Martin-Sampedro et al. 2012). However, after control and conventional sequences, although a decrease in the a* coordinate took place, an increase in the b* coordinate was observed, causing an increase in color (C*). The b* coordinate indicates the position between yellow and blue, and an increase in this coordinate means a shift to yellow. Therefore, the addition of an enzymatic pre-treatment in the bleaching sequences contributes to reduction of the yellowness of the resulting paper, which could be related to a smaller content of hexenuronic acids. A reduction in the amount of these acids present after LMS or xylanase treatments has also been reported elsewhere (Aracri and Vidal 2011; Eugenio et al. 2010; Oksanen et al. 2002; Valls and Roncero 2009; Martin-Sampedro et al. 2012). The lowest b* value was found when both xylanase and laccase were applied in sequence (XLEP), a result that is consistent with other studies (Martin-Sampedro et al. 2012 and Valls et al. 2010), where a greater reduction in the content of hexenuronic acids took place when xylanase was applied before LMS.

Comparing biobleached pulps (LEP, (X+L) EP, and XLEP) with their controls and a conventional bleached pulp, more lightness and less color were observed. These results indicate that an enzymatic pre-treatment enhances the subsequent bleaching process, providing pulps with better optical properties (Eugenio et al. 2011; Martin-Sampedro et al. 2011a; Valls and Roncero 2009), as was evidenced by the brightness results. Contrarily, no reduction in color (C*coordinate) has been reported when only laccase is used in the bleaching sequence (Fillat and Roncero 2010 and Martin-Sampedro et al. 2012). Furthermore, when both xylanase and laccase were used in our study, the optical properties improved, as compared to the pulp pre-treated only with laccase (LEP). When both enzymes were applied jointly in the same stage ((X+L) EP), the resulting pulp showed greater lightness than the pulp obtained when enzymes were applied in sequence (XLEP), although brightness values were similar in both cases. With respect to color properties, the application of the enzymes in sequence showed lower C* value, which

means that it was closer to the ideal neutral white (value 0). Therefore, it can be concluded that the application of xylanase and laccase in sequence brings about better optical properties, as it has been reported in previous occasions (Oksanen et al. 1997; Surma-Ślusarska and Leks-Stępień 2001; Martin-Sampedro et al. 2012). Irrespectively, more delignification (lower kappa number) takes place when both enzymes are combined in a single step, as it has been shown above.

Hydrogen Peroxide Consumption

Hydrogen peroxide consumption during the P stage was assessed in all experiments, and results are shown in Table 1. The smallest consumption was found in conventional EP bleaching, and not in biobleaching sequences. These findings are inconsistent with those obtained using *Eucalyptus globulus* kraft pulp (Eugenio et al 2010, 2011) but resemble those reported when using soda pulp from oil palm empty fruit bunches (Martin-Sampedro et al. 2012), which leads us to suggest that both the raw material and the pulping process can influence the hydrogen peroxide consumption. Furthermore, the conventional bleaching sequence provided pulps with lower brightness and higher kappa numbers, which could be related with the lower hydrogen peroxide consumption.

Comparing biobleaching sequences with their respective controls without addition of enzymes, an increase in hydrogen peroxide consumption was observed, except for the (X+L) EP sequence. However, as occurred in the conventional bleaching sequence, control sequences provided pulps with higher kappa numbers and lower brightness. The increase in hydrogen peroxide consumption when a second enzymatic/control stage was added (XcLcEP and XLEP sequences), without observing an increase in delignification, is also significant. Further analysis would be needed to clarify this result, since it could be related to a higher content of hexenuronic acids, which would increase the consumption of reagent in the bleaching process, and also in the kappa number determination, leading to overestimation of this parameter. But also, it could be caused by other factors, such as the residual lignin structure or the content of hemicelluloses, among others.

Table 1. Hydrogen Peroxide Consumption (%) Measured in the Different Bleaching Effluents after the LEP Sequence

Bleaching sequence	Hydrogen peroxide consumption (%)
LcEP	64.8
LEP	65.9
(X+L) EP	63.4
XcLcEP	81.0
XLEP	89.2
EP	56.9

To sum up, the biobleaching sequence that showed lower hydrogen peroxide consumption and provided pulps with lower kappa numbers was that in which xylanase and laccase were applied jointly in the same stage. Brightness was similar to that

observed when both enzymes were applied in sequence, and color properties were only slightly worse.

Handsheet Characterization

The influence of the different bleaching sequences in the mechanical properties of the paper was evaluated by generating handsheets from all bleached pulps obtained, and determining their tensile and tear indexes (Fig. 5).

The tensile and tear indexes observed were similar to those reported by other authors with olive tree residue pulps. Thus, López et al. (2003) studied the hydrogen peroxide bleaching of kraft pulps obtained from olive tree residue and reported tensile indexes slightly bigger (around 12.5 Nm/g), and tear indexes slightly smaller (around 0.55 Nm^2/g) than those observed in this study.

When LMS was applied, an increase in both the tensile and tear indexes was observed, compared to controls and unbleached pulps. Other authors have also reported an improvement of the mechanical properties of pulps treated with LMS, likely caused by the increased flexibility of the enzyme-treated pulps as a result of delignification and delamination of the cell wall (Herpoël et al. 2002; Moldes et al. 2010; You et al. 2008). However, when xylanase was applied, mechanical properties, especially the tensile index, were reduced in comparison to the LEP pulp. The tensile index is related to the linking capacity between fibers, so this reduction could be due to hemicelluloses (xylans) being lost during the xylanase treatment. Similar results have been reported elsewhere (Martin-Sampedro et al. 2011b) using a hemicellulose extraction method prior to pulping. The tensile index reduction observed when laccase and xylanase were applied in sequence, as compared to their use jointly, can be a consequence of the partial inactivation of the xylanase observed by Oksanen et al. (1997) when added in the same step as laccase.

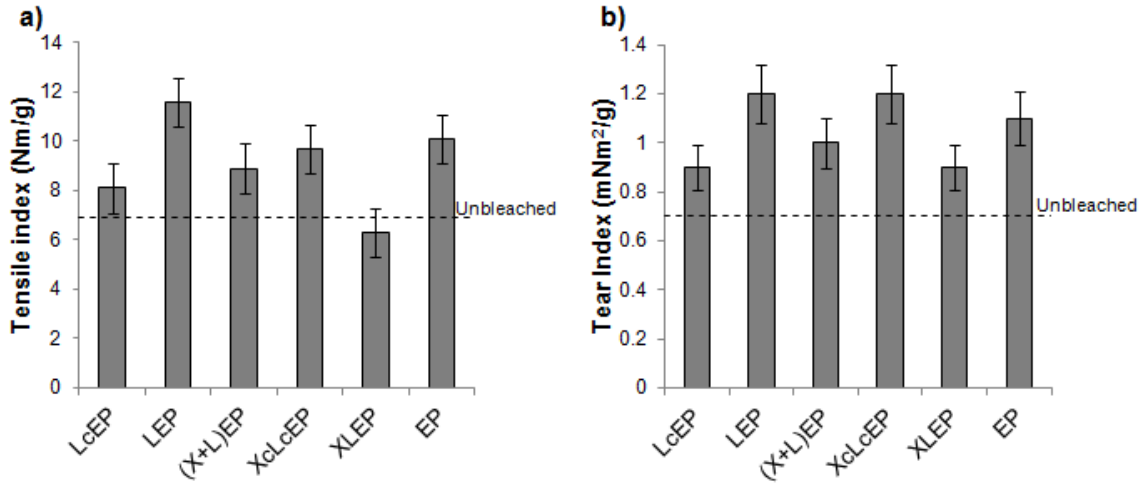

Fig. 5. Mechanical properties of handsheets from pulps obtained at the end of all the bleaching sequences assayed: a) tensile index; and b) tear index

Although some variations in the tear index were found across the different bleaching sequences, these were not as big as those observed in the tensile index. The tear index is related to the degree of fiber degradation of the pulp, so this result is

consistent with the similar viscosity found for all pulps. Herpoël et al. (2002) have reported no significant degradation of the cellulose matrix after the xylanase and laccase treatments, which is likely a specific feature of xylanase and laccase.

Accelerated Ageing

Accelerated ageing of bleached pulps was conducted in order to evaluate the effect of the enzymatic treatment on the stability of optical properties. Table 2 and Fig. 6, respectively show the values of brightness and color coordinates before and after accelerated ageing.

As it can be observed in Table 2, after accelerated ageing, all bleached pulps exhibited brightness reductions. These were greater in enzyme-treated pulps, compared to controls and conventionally bleached pulps. Similar results have been observed elsewhere (Martin-Sampedro et al. 2012), after accelerated ageing of pulp from oil palm empty fruit bunches. However, when eucalypt bleached pulp was used, the optical properties of LMS bio-treated pulps were more stable (i.e. less brightness reduction) during accelerated ageing, compared to controls (Cadena et al. 2010; Martin-Sampedro et al. 2011a). These authors attributed the smaller brightness reduction to a greater removal of hexenuronic acid in the enzymatic treatment. However, these researchers used eucalyptus kraft pulp with a higher hexenuronic acid content than olive soda pulp. This finding could be one of the reasons why the enzymatic treatment did not increase the optical stability of these soda pulps during accelerated ageing. Nevertheless, the hexenuronic acid content is not the only factor that can determine the different responses to accelerated ageing, but also lignin or hemicellulosic content (Vuorinen et al. 1999; Sevastyanova et al. 2005), which would explain the dissimilar behavior of different raw materials. Although less stable, enzyme-treated olive tree residue pulps showed higher final brightness than controls, even after accelerated ageing, with the highest brightness values being observed in bleached pulps treated with xylanase and laccase, either jointly or in sequence.

Table 2. Influence of the Accelerated Ageing Treatment in Brightness of Olive Residue Bleached pulps

Bleaching sequence	Brightness (% ISO)		
	Original[a]	Aged[b]	Reduction
LcEP	40.2	38.9	1.2
LEP	43.8	42.1	1.7
(X+L) EP	45.5	43.6	1.9
XcLcEP	39.6	38.8	0.8
XLEP	45.5	43.7	1.8
EP	39.9	38.6	1.3

[a] Before the accelerated ageing treatment
[b] After the accelerated ageing treatment

Figure 6a shows how the a* coordinate shifted to the right (from green to red) and the b* coordinate increased (from blue to yellow) in all bleached pulps after accelerated

ageing. Similar results have been reported with different raw materials (Cadena et al. 2011; Eugenio et al. 2011; Martin-Sampedro et al. 2012). Figure 6b shows an increase in color (C*) and a reduction in lightness (L*) in all pulps, after accelerated ageing, which is consistent with previous reports (Eugenio et al. 2011; Martin-Sampedro et al. 2012). Also after ageing, when both xylanase and laccase were applied, the color properties were better than with LEP, achieving the lowest C* value with the sequential application of these enzymes.

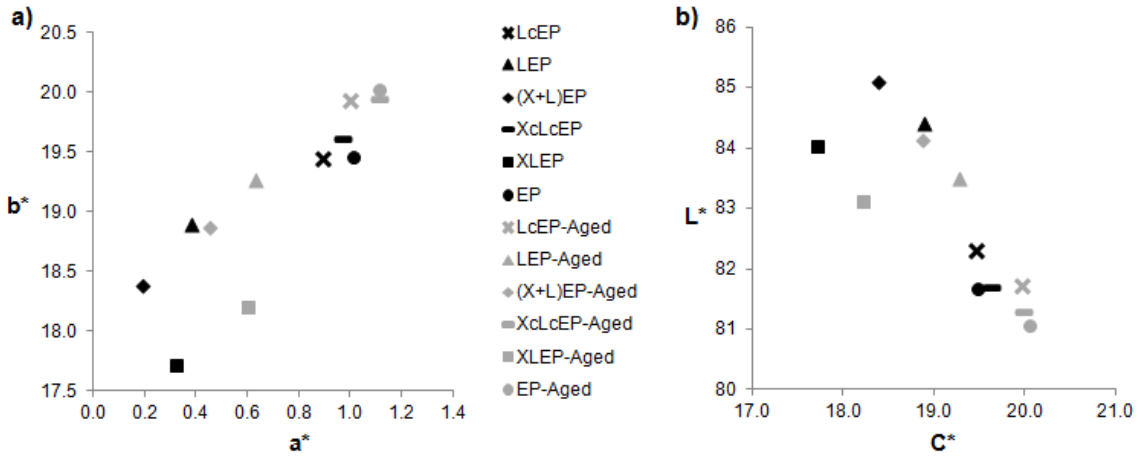

Fig. 6. This shows the influence of the accelerated ageing treatment in CIE L*a*b* and CIE L*C* color coordinates of the bleached pulps. Black and grey symbols represent pulps before and after accelerated ageing, respectively.

CONCLUSIONS

1. The application of an enzymatic pre-treatment with laccase and/or xylanase improved the bleachability of soda pulp obtained from olive tree pruning residue.
2. Xylanase boosted the laccase effect, probably by elimination of some xylan from the fiber surface, which favors access to laccase. When both enzymes were applied in the same stage, it resulted in more delignification, similar brightness, and less hydrogen peroxide consumption compared to a sequential application.
3. An improvement of CIE L*a*b* and CIE L*C* color coordinates was observed when a laccase-mediator system (LMS) stage was added to the bleaching sequence, and even more so when also xylanase was applied either jointly with LMS or in a previous stage.
4. Similar mechanical properties were found for all bleached pulps, although a reduction in the tensile index was observed when xylanase was applied, probably caused by hemicellulose removal.
5. Accelerated ageing caused a reduction in brightness and an increase in color coordinates in all pulps. Lower stability was found in biobleached pulps, compared with controls, although the best optical properties were observed in pulps treated with both xylanase and laccase, even after ageing.

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