

Refining and Urea Pretreatments to Enhance Biobleaching of Eucalyptus Kraft Pulp

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Some pretreatments that swell and/or open the structure of wood fibers could increase the effectiveness of a biobleaching process, allowing for an industrial application. To this end, a chemical pretreatment (urea, U), a physical pretreatment (refining, R), and their combinations (RU and UR) were optimized to evaluate and compare their enhancement of the LE biobleaching sequence (laccase-mediator treatment plus alkali extraction). The urea pretreatment before biobleaching (ULE) provided the highest delignification (37.5%) and the highest increase in brightness (6.1 points % ISO). As expected, adding a refining process before or after the urea pretreatment increased paper strength. However, when the refining was applied after the urea pretreatment (URLE), the delignification was higher than that obtained after RULE. Thus, URLE provided a similar Kappa number and an increase of 97%, 149%, and 98% in the tensile, tear, and burst indexes, respectively, compared with ULE treatment, but it had a reduction of 2.8 points (% ISO) in brightness, caused by the action of refining. Therefore, depending on the final use of the paper (which can require high optical properties or high strength), either ULE or URLE would be the optimal sequence.

Keywords: Refining; Urea; Laccase; Biobleaching; Eucalyptus kraft pulp

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INTRODUCTION

Environmental concerns have led to the displacement of conventional bleaching processes that use chlorine or its derivatives, giving way to bleaching processes that are more environmentally friendly, specifically elemental chlorine-free (ECF), and totally chlorine-free (TCF) processes. However, these processes also have disadvantages: ECF processes still carry chlorinated compounds in their effluents, and TCF processes lack selectivity.

An enzymatic stage before a traditional bleaching sequence could reduce problems associated with ECF and TCF processes. Several authors have shown that enzymatic pretreatment reduces the consumption of chemical bleaching reagents in subsequent bleaching stages without losing paper quality and generating less polluted effluents (Bajpai *et al.* 2006; Kapoor *et al.* 2007; Moldes and Vidal 2008; Valls and Roncero 2009; Eugenio *et al.* 2010, 2011a). One of the most valuable enzymes applied in biobleaching is laccase, as it is able to oxidize lignin derivatives and phenolic compounds using only molecular oxygen and low-weight molecules as mediators. This combination is called a “Laccase-Mediator System” (LMS) and was first described by Bourbonnais and Paice (1990). However, the application of LMS has some limitations, such as the high cost of the enzymes and the toxicity of the synthetic mediators, which prevent the

industrial full-scale application of the LMS in biobleaching sequences. To solve these problems, an intensive search for non-toxic mediators and new laccases for biobleaching purposes is being undertaken (Camarero *et al.* 2005, 2007; Eugenio *et al.* 2011a).

Alternatively, physical or non-oxidative chemical pretreatments can potentially improve the effectiveness of biobleaching and pave the way for its industrial application. As a physical treatment, refining is known to morphologically modify fibers in aqueous solution in a way that improves the mechanical properties of paper. Refining cuts the fibers and generates handsheets with more homogeneous properties. Refining also eliminates part of the external wall of the fibers, exposing the secondary wall to water, leading to hydration and swelling of the fibers. At the same time, fibrils generated on the surface of the fibers (fibrillation) improve the mechanical properties of the handsheets. Another effect of the combination of fiber cutting and fibrillation is a slow drainage of water; additionally, hydration and fibrillation together result in a specific area increase. Although refining has traditionally been implemented as a post-bleaching process, what has just been described suggests that its use as a pre-bleaching physical treatment could actually improve the outcome of the general process. Only a few studies (Lian *et al.* 2012) have been published in relation to this. These authors have demonstrated that the delignification and bleachability of wheat straw pulp was enhanced when using a mechanochemical process (refining at 7000 revolutions) prior to a biobleaching stage. However, no studies apart from that carried out in our laboratories (Eugenio *et al.* 2011b) have studied the effect of refining prior to bleaching using wood as a raw material.

Alternatively, as a non-oxidative chemical treatment, urea could improve biobleaching by inducing swelling or opening up of the fiber structure and the direct dissolution of lignin, hexenuronic acid (HexA), or chromophores. Urea has been used before with several purposes, including improving the dyeability of flax fibers (Sun *et al.* 2011), protecting cellulose in a chlorinated stage (Allan *et al.* 2000), combining with soda as an alternative method to the kraft pulping process (Cho *et al.* 2008), facilitating the isolation of lignin-carbohydrate complexes (LCCs) (Henriksson *et al.* 2007; Li *et al.* 2011), enhancing further enzymatic hydrolyses (Wang *et al.* 2011; Mohsenzadeh *et al.* 2012), and improving hydrogen peroxide bleaching when urea was added at the same time as the chemical bleaching reagent (Cai 2005; Liu *et al.* 2006; Zhao *et al.* 2008; Zhang *et al.* 2013). However, until now, only previous studies carried out in our laboratories (Eugenio and Villar 2012) have demonstrated that urea pretreatment can enhance a subsequent biobleaching process. Nevertheless, a deeper study of the optimization of these pretreatments is needed to scale up these processes. Moreover, the combination of urea and refining pretreatments could increase their effects in the subsequent biobleaching process.

Therefore, the main goal of this work was to apply an optimal physical pretreatment (refining, R), an optimal non-oxidative chemical pretreatment (urea, U), and their combinations (UR and RU) over an industrial unbleached eucalyptus kraft pulp to evaluate and compare their effects on a subsequent biobleaching process. The biobleaching sequence consists of an enzymatic treatment using the laccase produced by the ligninolytic fungus *Pycnoporus sanguineus* in conjunction with a natural mediator (acetosyringone), followed by an alkaline extraction to assure a complete removal of the attacked lignin.

EXPERIMENTAL

Raw Material

Industrial-grade unbleached *Eucalyptus globulus* kraft pulp was provided by La Montañanesa pulp mill (Torraspapel - Lecta Group, Spain). Its Kappa number, brightness, and viscosity were 14, 35 % ISO, and 1166 mL/g, respectively.

Chemicals

All chemicals were reagent-grade. The NaOH, urea, MgSO₄, and H₂O₂ were obtained from Panreac Química SAU (Barcelona, España); 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonate (ABTS) was purchased from Roche (Madrid, Spain); diethylene triamine pentaacetic acid (DTPA) was purchased from Merck (Barcelona, Spain), and acetosyringone (4-hydroxy-3,5-dimethoxyacetophenone) was purchased from Sigma-Aldrich (Madrid, Spain).

Optimization of the Urea Pretreatment (U)

Urea treatments were carried out over unbleached *E. globulus* kraft pulp in polyethylene bags submerged in a thermostatic bath at 80 °C. A 33-factorial design (27 experiments) was used to determine the optimal conditions. The independent variables considered were urea concentration (2, 5, or 8 M), treatment time (2, 25, and 48 h), and consistency (5, 10, or 15 %).

The goal of this pretreatment is to improve a subsequent bleaching process. Therefore, after the urea pretreatments and before determining the properties of the obtained pulps, a standard chemical bleaching with hydrogen peroxide (P) was carried out.

The operational conditions were as follows: 2% H₂O₂ over dry pulp (odp), 1.5% NaOH odp, 1% DTPA odp, 0.2% MgSO₄ odp, 5% consistency, and 90 °C for 90 min. A control without any urea pretreatment was also tested. Residual hydrogen peroxide was analyzed in all bleaching effluents by standard titration.

Bleaching with hydrogen peroxide was followed by determination of the Kappa number (UNE 57034), viscosity (UNE 57-039-92), and brightness (UNE 57062) in all pulps. These results provided the basis for selecting the optimal conditions for the urea pretreatment.

Optimization of the Refining Pretreatment (R)

The unbleached *E. globulus* kraft pulp was refined at 1000, 2000, and 3000 revolutions using a PFI laboratory mill, according to the standard UNE-EN ISO 5264-2. To evaluate the effect of this pretreatment on a subsequent bleaching process, a standard chemical bleaching with hydrogen peroxide (P) was carried out. The operational conditions were as follows: 2% H₂O₂ odp, 1.5% NaOH odp, 1% DTPA odp, 0.2% MgSO₄ odp, 5% consistency, and 90 °C for 90 min. Residual hydrogen peroxide was analyzed in all bleaching effluents by standard titration. A control without the refining pretreatment was also tested.

Finally, Kappa number (UNE 57034), viscosity (UNE 57-039-92), and brightness (UNE 57062) were determined in all bleached pulps, and the optimal conditions for the refining pretreatment were selected.

Refining and Urea Pretreatments to Enhance the Biobleaching Process

Combination of treatments (U), (R), (RU), and (UR)

Urea (U) and/or refining (R) pretreatments were applied before the LMS treatment (L), followed by an alkaline extraction (E), to test their effect on the biobleaching process. The operational conditions used in these pretreatments were those selected as optimal in previous sections.

Pretreatments were tested alone and combined; the sequences carried out were as follows: ULE, RLE, RULE, and URLE.

Laccase-mediator system treatment (L)

The laccase used in this work was obtained in our lab from the ligninolytic fungus *Pycnoporus sanguineus*, as described by Eugenio *et al.* (2009). The optimal conditions for its application in a biobleaching process have been studied previously (Eugenio *et al.* 2010).

Assays were performed using 35 g of dry pretreated pulp in 500-mL reactors and mixed with laccase and mediator. Oxygen was added until reaching a pressure of 6 kg/cm². Then, the reactors were submerged in a thermostatic bath. Consistency, reaction time, laccase dose, and mediator concentration during the laccase treatment were fixed at 10%, 1 h, 2.4 UA laccase/g odp, and 0.05 mmol acetosyringone /g odp, respectively. Here, 1 U of laccase is defined as the amount of laccase required to convert 1 μmol/min of ABTS to its cationic radical (0.1 M acetate buffer, pH 5.2, 24 °C). Temperature and pH were set at 40 °C and pH 3 (citrate-phosphate buffer, 0.1 M), as these conditions are known to maximize the enzymatic effects on phenolic units of lignin when using *Pycnoporus sanguineus* laccase (Eugenio *et al.* 2010). Moreover, some drops of 0.05% (v/v) polyoxyethylene (20) sorbitan monooleate (Tween 80) were added to all assays to improve the interaction between enzyme and substrate.

Alkaline extraction (E)

After the enzymatic treatment, pulps were washed with distilled water until reaching neutral pH and air dried at room temperature. Then, an alkaline extraction was carried out to ensure the complete removal of the lignin attacked during the enzymatic treatment. This extraction was performed under a final NaOH concentration of 1.5% odp, 5% consistency, and 90 °C for 120 min. Afterward, the pulps were washed again with distilled water until reaching neutral pH and air dried at room temperature.

Pulp and handsheet characterization

The unbleached pulp and treated pulps obtained after each step of the biobleaching sequences were characterized in terms of their Kappa number (UNE 57034) and brightness (UNE 57062). Furthermore, handsheets were formed from bleached pulps at the end of the sequences in accordance with UNE-EN ISO 5269-2, and the tensile, tear, and burst indexes were determined (UNE-EN ISO 5270).

RESULTS AND DISCUSSION

Optimization of the Urea Pretreatment

Table 1 summarizes the experiments carried out to optimize the urea pretreatment, as well as the Kappa number, viscosity, and brightness of the bleached pulp obtained

after the P stage. Hydrogen peroxide consumption was also evaluated; results are included in Table 1. A control assay (named control P) in which only the hydrogen peroxide stage was carried out (without urea pretreatment) was also tested.

Table 1. Optimization of the Urea Pretreatment: Operational Conditions, Hydrogen Peroxide Consumption and Kappa, Viscosity, and Brightness of the Bleached Pulps Obtained

Experiment (UP)	Urea Conc. (M)	Reaction time (h)	Consistency (%)	H ₂ O ₂ consump. (%)	Kappa number	Viscosity (mL/g)	Brightness (% ISO)
Control (P)	-	-	-	52.8	9.3	1132	51.6
1	8	48	5	30.2	8.6	1020	49.9
2	8	48	10	28.3	8.8	1061	46.7
3	8	48	15	26.1	8.2	995	47.9
4	8	25	5	33.2	8.4	1020	51.4
5	8	25	10	28.8	8.3	1005	51.7
6	8	25	15	25.6	8.0	1009	49.5
7	8	2	5	40.8	8.1	1099	53.2
8	8	2	10	34.1	8.0	1222	55.3
9	8	2	15	52.5	7.5	1044	56.3
10	5	48	5	18.4	8.6	1024	47.6
11	5	48	10	25.3	8.9	1057	46.7
12	5	48	15	22.1	8.5	1024	48.6
13	5	25	5	34.4	8.5	1100	50.5
14	5	25	10	28.6	8.6	1066	50.6
15	5	25	15	30.7	8.5	1030	49.6
16	5	2	5	41.3	8.2	1109	54.5
17	5	2	10	50.8	8.0	1102	55.0
18	5	2	15	50.3	8.2	1077	55.1
19	2	48	5	25.5	8.7	1075	48.9
20	2	48	10	28.1	8.5	1028	49.6
21	2	48	15	30.9	8.8	1055	51.2
22	2	25	5	33.0	8.9	1086	50.3
23	2	25	10	33.3	8.5	1078	52.1
24	2	25	15	33.4	8.1	1050	51.8
25	2	2	5	47.2	8.4	1061	53.9
26	2	2	10	49.0	8.0	1108	54.9
27	2	2	15	52.1	8.3	1002	54.6

A reduction in Kappa number across all cases compared to the control pulp (between 4.3 and 19.4%) was observed. Allan *et al.* (2000) also observed a 7% reduction in Kappa number when urea was used as cellulose protection in the chlorination stage of an ECF bleaching sequence. In their case, urea was added during the bleaching stage and

not as a pretreatment stage. This could be the reason why more delignification was observed in this experiment (19.4% in the best case), as urea causes fiber swelling (Henriksson *et al.* 2007) and leads to an easier penetration of oxidative chemicals into the material in a subsequent treatment. On the contrary, Van Tran (2000) did not find higher delignification when urea was used in an oxygen bleaching stage.

The highest delignification was observed when the urea concentration and consistency were the highest (8 M and 15%, respectively) and when the reaction time was the lowest (2 h). Cho *et al.* (2008) have observed that the alkalinity grade is an important factor in delignification when urea was added in the soda pulping of oak wood. Thus, the addition of urea in low alkali charges retarded the delignification rate in soda pulping compared to the control (soda pulping without urea addition). However, delignification was significantly enhanced with high urea charges (implying high alkalinity). The authors confirmed that these results were attributed to the high alkalinity, not the urea addition. Therefore, this statement could justify why the highest delignification was observed when a urea concentration of 8 M and 15% consistency was used, as this combination corresponds to the experiment with the highest alkalinity grade (alkali charge per gram of pulp). However, when urea concentration was lower than 8 M (5 or 2 M), no significant differences in delignification were found with varying consistencies (5, 10, or 15%). This result could indicate that higher alkalinity grade (such as that reached with 8 M and 15% consistency) is required to ensure higher delignification.

Regarding time, the highest delignification was observed at the shortest reaction time assayed (2 h) and was independent of the urea concentration and consistency used. Cho *et al.* (2008) also observed higher delignification at a short length of time when urea was used in a soda cooking process. Thus, they reported an increase in delignification from 85.3% to 91.8% when the time was reduced from 150 to 90 min.

On the other hand, urea pretreatment does not cause strong cellulose degradation. This could be due to two simultaneous and opposing effects that can compensate the viscosity values: (i) the urea treatment could break down the carbohydrates and decrease the viscosity (Cho *et al.* 2008); (ii) the extraction of the short chains of polysaccharides, mainly hemicelluloses, during this stage could cause an increase in viscosity (Eugenio *et al.* 2011b) and also a slight reduction in pulp yield. Contrarily, Allan *et al.* (2000) found an increase in viscosity (around 40%) when urea was used as cellulose protection in the chlorination stage of an ECF bleaching sequence.

Regarding pulp brightness, it can be observed that the highest brightness (56.3% ISO compared to 51.6% ISO for control) was observed when the urea concentration and consistency were the highest (8 M and 15%, respectively), and the time was the lowest (2 h). This result is consistent with Kappa number results, as this experiment also provided the highest delignification. Zhao *et al.* (2008) and Zhang *et al.* (2013) have also observed an increase in brightness (2.6 and 3 points % ISO more compared to their controls, respectively), although they used urea during the hydrogen peroxide bleaching stage and not as a pretreatment.

In addition, although hydrogen peroxide consumption decreased in all assays compared to the control (consistent with results found by Cai (2005) and Liu *et al.* (2006) when they used urea in the hydrogen peroxide bleaching stage), longer urea treatments were associated with lower hydrogen peroxide consumptions and brightness decreases. Eugenio *et al.* (2011b) observed an increase in condensed lignin after a 2-h urea treatment. Therefore, a longer urea treatment could produce a higher amount of condensed

lignin, which is more difficult to bleach. This fact would explain the lower hydrogen peroxide consumption and brightness at longer reaction times.

Based on the final results, it seems that the best conditions for urea pretreatment to enhance the subsequent bleaching step were as follows: a high urea concentration (8 M), a high consistency (15%), and a short treatment time (2 h). At these conditions, the highest alkalinity grade was reached, which would not only assure a high pH at the end of the treatment, but would also avoid lignin precipitation, which is more difficult to bleach, apart from giving a dark color to the pulp. The lowest Kappa number (7.5 compared to 9.3 for the control pulp) and the highest brightness (56.3 compared to 51.6 % ISO for control pulp) obtained without degrading the pulp viscosity were observed at these selected conditions. However, although the hydrogen peroxide consumption was not the lowest in this experiment, there was a slight decrease in this parameter compared to the control, achieving higher delignification. This involves a better use of the chemical reagents during the hydrogen peroxide bleaching process brought about by the urea pretreatment.

Optimization of the Refining Pretreatment

Kappa number, viscosity, and brightness of the bleached pulps after refining (R1, R2, and R3), a P stage, and hydrogen peroxide consumption are shown in Table 2. A control assay in which the unbleached pulp was not subjected to a refining pretreatment before the P stage was also evaluated.

Table 2. Optimization of the Refining Pretreatment: Operational Condition, Hydrogen Peroxide Consumption, and Kappa, Viscosity, and Brightness of the Bleached Pulps Obtained

Sample	PFI revolutions	H ₂ O ₂ consump.(%)	Kappa number	Viscosity (mL/g)	Brightness (%ISO)
Control (P)	-	72.8	9.3	1132	51.6
R1P	1000	91.8	9.5	995	47.5
R2P	2000	87.8	8.9	996	47.0
R3P	3000	87.7	8.9	991	47.0

The highest delignification was obtained with a refining pretreatment of 2000 or 3000 revolutions (4% higher than the control P experiment in both cases). Refining at 1000 revolutions did not result in an increase in delignification compared to the control pulp. Therefore, 1000 revolutions do not seem to be enough to effectively open the fibers and make the subsequent bleaching process easier.

Lian *et al.* (2012) have also observed higher delignification (10%) when a refining treatment of 7000 revolutions was applied over wheat straw pulp prior to the bleaching process compared to their control (without refining pretreatment). The authors attributed this effect to the repeated changes in the shape of the cell wall in the course of refining. The primary wall (P) and the S1 layer were exploded, and the fiber was cut to some extent. Because the P and S1 layers contain a higher proportion of lignin, this lignin was exposed outside after refining, which helps to promote the reaction between the bleaching agent (such as the hydrogen peroxide used in this study) and lignin, resulting in a higher degree of delignification. The delignification obtained by Lian *et al.* (2012) was

higher than that obtained in our case; however, a different raw material (wheat straw, which has a more ready accessible tissue structure than *E. globulus*) and higher revolutions (7000 instead of 2000) were applied during the refining process.

The slight decrease in viscosity observed after all refining stages was probably the result of the generation of short carbohydrate chains during refining. Lian *et al.* (2012) also found that the refining had little effect on viscosity.

The pulps were also noticeably darkened after each refining stage (there was a decrease of approximately 4 points % ISO compared to the control pulp); this was likely due to the formation of new chromophores, as demonstrated by Eugenio *et al.* (2011b). Nevertheless, when comparing the brightness values measured after refining but before the hydrogen peroxide stage (R1, R2, and R3 samples, data not shown) with those obtained after the hydrogen peroxide bleaching (R1P, R2P, and R3P samples), greater increases were found when a more extensive refining pretreatment was carried out. This is due to the fact that the physical treatment opens the fibers, exposing more of the pulp surface to be bleached in the next step (increase of 16.6, 17.1, 18.7, and 21.2 points % ISO for control P, R1P, R2P, and R3P, respectively). Lian *et al.* (2012) also found a decrease of around of one point % ISO in brightness after a bleaching sequence in pulps that had been pretreated with a refining process. This decrease was much lower than that observed in our case, although it has been mentioned before that a different raw material and refining process had been used by these authors. They explained that the residual lignin can be oxidized by the bleaching reagent; hence, the absorption coefficient of pulp increased and brightness decreased. These effects appeared to offset the increase due to the lowered lignin content in the case of pulp refined at 2000 and 3000 revolutions (where slight delignification was observed).

All pulps consumed almost the whole amount of hydrogen peroxide initially added, except for the control pulp. The reason behind this finding could be that after refining, more fiber surface is exposed to the bleaching reagent, which increases accessibility and consequently increases reagent consumption, even at 1000 revolutions, although no delignification was observed. When the refining was more extensive (2000 and 3000 revolutions), similar hydrogen peroxide consumption resulted in higher delignification thanks to a more efficient use of the reagent. However, at the same delignification degree, more chemical reagent could be saved, as Lian *et al.* (2012) have demonstrated.

In conclusion, similar pulp properties and similar hydrogen peroxide consumption were observed in pulps subjected to 2000 and 3000 revolutions. Furthermore, these pretreatments provided higher delignification after the P stage than the control pulp and the pulp subjected to a milder refining pretreatment (1000 revolutions). Therefore, the optimum grade of refining would be 2000 revolutions, as refining at 3000 revolutions involved higher energy consumption and did not show better results.

Effect of the Urea and the Refining Pretreatments on Biobleaching

Once U and R pretreatments were optimized, it was observed that the U pretreatment yielded greater enhancement of chemical bleaching than refining pretreatment (7.5 vs. 8.9 Kappa number and 56.3 vs. 47.0 % ISO brightness for UP and RP, respectively). After this, the effect of these pretreatments and their combinations on a biobleaching sequence was studied. Here, biobleaching consisted of an enzymatic treatment with LMS, followed by an alkaline extraction to remove the attacked lignin (LE). Thus, the pretreatment applied before the biobleaching sequence was composed of

urea and refining pretreatments separately (ULE and RLE), their combination in the same sequence (URLE and RULE), and a control without pretreatments (LE).

As has been mentioned before, an enzymatic treatment can be used as an alternative to avoid the problems associated with ECF and TCF processes. Thus, after the LE sequence, a 26.3% reduction in the Kappa number was achieved from the initial pulp, as seen in Fig. 1. Similar delignification has been observed by Eugenio *et al.* (2010) after the same sequence (LE). These authors reported that the LMS pretreatment not only delignifies but also modifies the residual lignin into a form that is easier to remove in the subsequent alkaline extraction. Therefore, an alkaline extraction after the enzymatic treatment is necessary to evaluate the global effect of the LMS treatment. Moreover, if a greater delignification were necessary, more bleaching stages could be added at the end of this sequence. Thus, Eugenio *et al.* (2010) have observed that delignification increases up to 49.2% when a hydrogen peroxide stage is added after the LE.

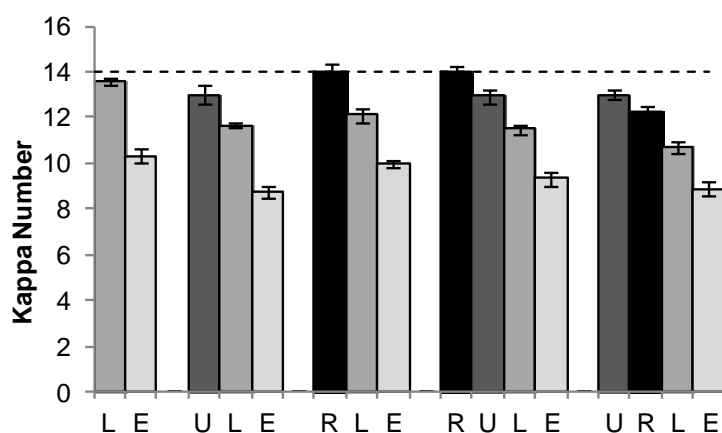


Fig. 1. Kappa number of the unbleached pulp and after each stage of the different biobleaching sequences

To improve the delignification obtained by the enzymatic treatment, different pretreatments such as urea or refining could be used. Thus, compared to the control sequence (LE), all sequences in which a urea or refining pretreatment or their combination was applied provided pulps with lower Kappa numbers (between 3.4 and 15% lower, depending on the assay). Therefore, both refining and urea pretreatments enhanced the ensuing biobleaching process. Eugenio and Villar (2012) found an increase of 22% in the delignification of pulps pretreated with the same pretreatments and then subjected to a standard chemical bleaching process (RUP sequence) compared to their control (P sequence). Therefore, it seems that urea and refining pretreatments enhanced the chemical bleaching more than the enzymatic bleaching. However, if a final P stage is added after the RULE treatment, higher improvements can be achieved.

It should be noted that although no delignification was observed after refining in the first stage, this process opens up the pulp structure and increases the effectiveness of the subsequent laccase process, as can be observed in the RLE sequence (Fig. 1). Similar results have been found by Eugenio *et al.* (2011a), although they used a bacterial laccase instead of the fungal laccase that was used here. However, although these authors observed a thermal decomposition of HexA structures after refining, which would interfere with the Kappa number determination (Sevastyanova 2005; Valls 2008), this effect was not observed in our experiment, as the same Kappa number was measured after applying the refining process.

On the other hand, delignification observed after the ULE sequence (37.5%) was greater than with the RLE sequence (28.9%). This result was expected because the urea treatment not only swells up the fibers (Henriksson *et al.* 2007; Li *et al.* 2011), favoring subsequent treatment, but also causes delignification in the pulp due to the alkalinity of the process, as Cho *et al.* (2008) have demonstrated. Similar results were found when the urea and the refining pretreatments were optimized for chemical bleaching in the previous sections.

The combination of refining and urea as pretreatments did not improve delignification compared to the ULE sequence. ULE provided pulp with a Kappa number of 8.8, while RULE and URLE yielded pulps with a higher or similar Kappa number (9.3 and 8.9, respectively). From these results, it can be concluded that the application of a urea pretreatment as the first stage in the bleaching sequence provided the best results, increasing delignification up to 15.2% compared to the control sequence (LE).

Figure 2 shows the brightness evolution during the different bleaching sequences. An increase in brightness was observed after each stage, the only exception being the refining pretreatment. Darkening of the pulps after this process has been explained by Eugenio *et al.* (2011b), who by means of a thioacidolysis-SEC analysis observed a higher amount of chromophores in the refined pulps compared to the control pulp.

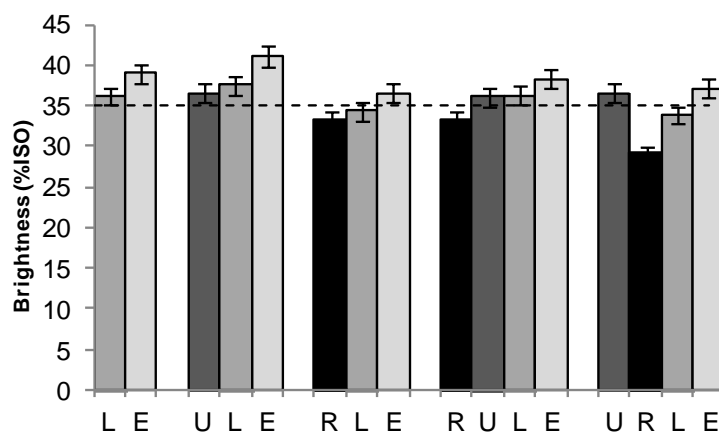


Fig. 2. Brightness of the unbleached pulp and after each stage of the different biobleaching sequences

Final brightness in the control biobleaching sequence (LE) was the same as that reported by Eugenio *et al.* (2010) (39 % ISO) after an equivalent sequence and greater than that reported without any enzymatic treatment (34 % ISO for E stage). When a urea pretreatment was included in the enzymatic sequence, greater increases in brightness were achieved (41 % ISO) (Fig. 2) as a consequence of urea acting on the pulp structure and on the delignification. On the other hand, the lowest brightness was observed with the RLE sequence, which is consistent with the aforementioned reduction in brightness after refining. Overall, sequences that included a refining stage, with or without a U pretreatment, resulted in lower brightness compared to a similar sequence without the R stage. It can be also observed that among all sequences that include a refining stage, the RULE sequence showed the highest brightness. The reason for this has been explained by Eugenio *et al.* (2011b), who showed that urea can remove some of the chromophoric groups formed during the refining treatment. Thus, RULE showed the highest brightness after the ULE sequence (38 vs. 41 % ISO, respectively).

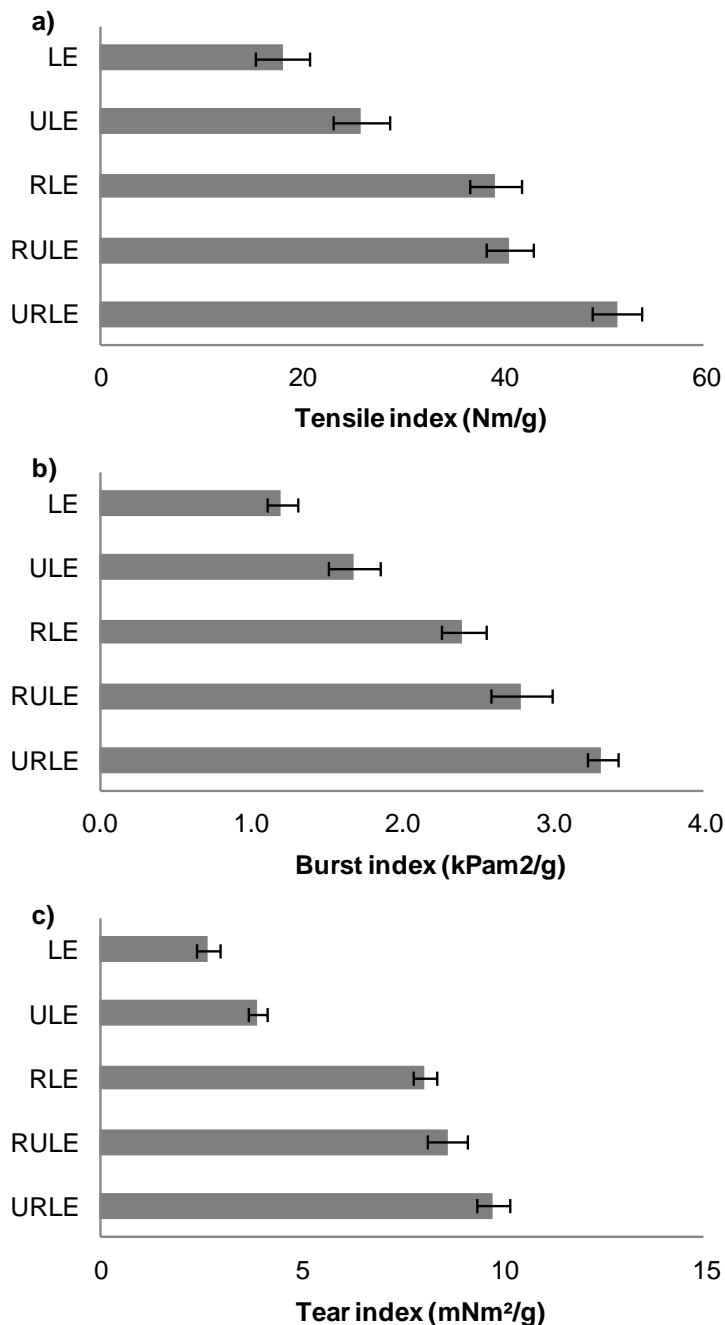


Fig. 3. Tensile (a), burst (b), and tear (c) indexes of handsheets formed from bleached pulps after the different biobleaching sequences

Finally, the mechanical properties of the handsheets formed were measured at the end of the sequences, and the results are shown in Fig. 3. It can be observed that when a standard biobleaching LE sequence was applied (control pulp), the tensile, tear, and burst indexes decreased compared to those of unbleached pulp (18.1 vs. 32.4 Nm/g; 2.7 vs. 4.1 mNm²/g, and 1.2 vs. 1.8 kPam²/g, respectively). Nevertheless, this reduction in mechanical strength would be greater if conventional bleaching without enzymatic treatment were to be applied, according to several authors (Herpoël *et al.* 2002; You *et al.* 2008; Moldes *et al.* 2010; Martin-Sampedro *et al.* 2012). These authors have reported an

improvement of the mechanical properties of bleached pulps treated with LMS compared to conventional bleaching, which is likely caused by the increased flexibility of the enzyme-treated pulps as a result of delignification and delamination of the cell wall.

Additionally, greater improvements of mechanical strength could be achieved if a urea and/or refining pretreatment were included in the biobleaching sequence (Fig. 3). Thus, when a urea pretreatment was applied, the tensile, tear, and burst indexes increased up to 43%, 47%, and 40%, respectively, compared to the control pulp (LE). This finding is likely the consequence of increased cross-linking between swollen fibers. Although they used pulping instead of bleaching, Cho *et al.* (2008) also found an increase in mechanical properties (21%, 22%, and 33% in the tensile, tear, and burst indexes, respectively) in pulp obtained using soda pulping with the addition of urea compared with pulp obtained with standard soda pulping.

As expected, refining resulted in an increase of all indexes (116%, 204%, and 100% in the tensile, tear, and burst indexes, respectively, compared to the LE pulp). As previously mentioned, refining is known to cause fibrillation, in which fibrils are generated on the surface of the fibers and become intertwined with other fibrils from adjoining fibers, improving the mechanical properties of the handsheets. Lian *et al.* (2012) also found an increase in the mechanical properties (4%, 4.5%, and 6.5% in the breaking length and tear and burst indexes, respectively) when refining was used as a pretreatment in a biobleaching sequence apart from the reduction in chemical consumption to reach similar bleachability of the pulp.

When both pretreatments were carried out in sequence, higher tensile, tear, and burst indexes were achieved, but it was when urea was applied before refining (URLE) that the highest indexes were found (increases of 183%, 267%, and 177% for the tensile, tear, and burst indexes, respectively, compared to the LE pulp). The improvement observed with this sequence, compared to RULE, could be a consequence of the pre-swelling of the fibers (during U treatment), which would enhance subsequent refining and produce better mechanical properties. Moreover, the urea treatment is known to result in a slight reduction in HexA (Eugenio *et al.* 2011b). This reduction before refining allows for a better paper with specific strength-related properties and a reduced Schopper-Riegler index without using additional refining energy suggesting that HexA influence fiber-fiber cohesion (Cadena *et al.* 2010).

All these results indicate that, as far as the mechanical properties are concerned, URLE is the most efficient sequence. The tensile, tear, and burst indexes increased 58%, 137%, and 84%, respectively, from the original unbleached pulp. These increases are mainly an effect of the refining process, as, when compared with the refined unbleached pulp, all the mechanical properties decreased (8%, 2%, and 19% in the tensile, tear, and burst indexes, respectively). However, these reductions in paper strength after the URLE sequence are smaller than those observed in a standard bleaching sequence (Martin-Sampedro *et al.* 2012). This is attributable mainly to the urea pretreatment but also to the biological treatment.

CONCLUSIONS

1. After U and R optimization, the U pretreatment resulted in the greatest enhancement of chemical bleaching (7.5 vs. 8.9 Kappa number and 56.3 vs. 47.0 % ISO brightness for UP and RP, respectively).

2. These pretreatments and their combinations also boosted the LE biobleaching sequence.
3. Adding urea as the first stage in the sequence (ULE and URLE) resulted in the greatest increase in delignification. However, URLE yielded better mechanical properties (increase of 97%, 149%, and 98% in the tensile, tear, and burst indexes, respectively) but yielded a decrease of 2.8 points % ISO in brightness compared to the ULE sequence.
4. Depending on the final use of the paper (which can require high optical properties or high strength), either ULE or URLE would be the optimal sequence.

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