LIGNIN BLENDS WITH POLYURETHANE-CONTAINING LACTATE SEGMENTS. PROPERTIES AND ENZYMATIC DEGRADATION EFFECTS

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A series of lignin-polyurethane blends were synthesized from biodegradable poly(ester-urethane) elastomers containing lactate segments and lignin (flax/soda pulping) by mixing their dimethylformamide solutions. The resulting films were subsequently treated with fungal peroxidase and laccase, to compare the degradative potential of these enzymes on the natural and synthetic blend components. Lignin addition was found to substantially improve the structural organization of polyurethanes and, hence, the mechanical properties of blends. Also, lignin determines a consistent reduction of mass loss during thermal decomposition, especially in the case of previously enzyme-treated samples. The attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra showed that both lignin and polyurethane were affected by the oxidative enzymatic treatment, at least on film surface. Polyurethane degradation was mainly related to the chain scission of polyester and lactate segments, being strongly influenced by lignin addition and enzyme type, and accompanied by significant changes in the subsequent thermo-mechanical behavior.

Keywords: lignin, polyurethane, blends, peroxidase, laccase, tensile strength, thermal decomposition

INTRODUCTION

The complex upgrading of natural products, together with the extended synthesis and use of biodegradable materials and reduction of environmental impact generated by traditional plastic materials represent most important research topics, which have drawn a constantly growing interest in the last decade.[1,2] In this context, the use of lignin for obtaining new classes of biodegradable engineering plastics is highly motivated by the abundance of this natural renewable resource and by its advantageous properties, such as relatively numerous reactive functional groups, great variety of modification options, moderate biodegradability, good adhesive, adsorption and solution properties, and compatibility with several basic chemicals.[3] On the other hand, polyurethane versatility offers the potential for preparing a wide class of materials, including multicomponent polymeric systems, like composites, blends and interpenetrating polymer networks with polymers from natural sources.[4,5]

Traditional polyurethanes could present certain degrees of biodegradability, which vary as a function of composition, processing and environmental conditions, however, their biodegradation rates are considerably slower and may be improved by incorporation of biodegradable structural units in the main chain.[6,7] One of the most frequently reported biodegradable insertions involves the use of low molecular polylactates obtained through the polycondensation of lactic acid with different diols as raw materials for polyurethane synthesis.[8,9] Lignin and its derivatives were also used either as polyolic precursors for polyurethanes,[10] or for the preparation of lignin-polyurethane blends and composites.[11,12]

As previously reported,[13] the addition of no more than 10% (w/w) lignin imparts strength and biodegradability to polyurethane

elastomers, together with a lower elasticity and decomposition temperature. In the present work, biodegradable polyurethane containing lactate segments has been synthesized for studying the effect of lignin blending on the stability and thermo-mechanical behavior of the resulted materials. Furthermore, since such materials are well-suited for outdoor and packaging applications, their responses to oxidative enzymatic environments were tested. Two fungal oxidases, characterized by distinct mechanisms of action: peroxidase (*Aspergillus sp.* NS 51004) and laccase (*Aspergillus sp.* NS 51003), have been selected as enzymes.

It is generally known that peroxidases are hemoproteins that catalyze the oxidation of various substrates containing electron acceptor groups (*i.e*. phenol, phenyl, amino, carboxyl) in two one-electron steps reactions, with the involvement of a peroxyl molecule (*i.e.* H_2O_2, organic peroxide),[14,15] whereas laccases are copper metalloproteins, which catalyze the one-electron oxidation of a wide range of substrates (*i.e*. phenol and polyphenol derivatives, aromatic amines) with the parallel four-electron reduction of molecular oxygen to water, and without producing hydrogen peroxide.[16] The radicals formed on the macromolecular substrate chain do not determine only a cascade of enzymatic and non-enzymatic degradation reactions, but may also participate, especially in the presence of laccase, at crosslinking and polymerization processes.[17] Although numerous reports discuss lignin degradation under the action of such enzymes,[18] no data related to their degradative potential on polyurethane materials are available. Consequently, a comparative analysis of some of the effects of enzymatic pretreatment on the surface structure and thermo-mechanical behavior of pure and lignin-blended polyurethane containing lactate segments was performed.

EXPERIMENTAL

Materials

Lignin, kindly provided by Granit SA (Switzerland) as an industrial product of flax/soda pulping (PF 3035 type, apparent density – 0.4 g/cm^3, humidity – 6.2%, mineral ash – 7.36%, phenolic OH – 1.58 mmol/g, COOH – 1.75 mmol/g), was used without further modifications.

4,4'-diphenylmethane diisocyanate (MDI; 99.2%), ethylene glycol (EG; 98%), adipic acid (99%), diethylene glycol (DEG; 99%) and racemic lactic acid (90%) were obtained from Sigma-Aldrich, and used as received.

Fungal peroxidase (E.C. 1.11.1.7, NS 51004, 10000 POXU/g) and laccase (E.C. 1.10.3.2, NS 51003, 1000 LAMU/g) isolated from *Aspergillus* sp. were provided by Novozymes AS, Denmark. The H_2O_2 solutions (~ 30%) were purchased from Merck. All other reagents used were of analytical grade.

Polyurethane synthesis

The biodegradable polyurethane elastomer containing lactate segments (PLU) was synthesized as described previously.[19,20]

In the first step, diethylene glycol lactate (DEGL) was obtained through direct condensation of racemic lactic acid (1 mol) and DEG (1.66 mol) for 2 h, at a temperature of 160 ^oC and a pressure of 5 mmHg, with continuous elimination of water. The synthesized DEGL had a number-average molecular weight of 180, a hydroxyl number of 622 mg KOH/g, and an acidity index of 0.022 mg KOH/g compound.

Furthermore, poly(ethylene adipate) (PEA, number-average molecular weight – 2000, hydroxyl number – 56 mg KOH/g, and acid number – 0.2 mg KOH/g) was obtained through condensation of adipic acid and ethylene glycol (1:1.1 molar ratio), vacuum-dried at 80 °C for 1 h, and subsequently reacted with 4,4'-diphenylmethane diisocyanate (MDI). The resulted polyurethane prepolymer was then reacted with ethylene glycol and diethylene glycol lactate, used as chain extenders (PEA:MDI:EG:DEGL = 1:6:4:1 molar ratio), until the resulted polymer was fully free of NCO groups. Polyurethane synthesis was carried out in DMF 40 wt% at 60-65 °C for 4 h, without addition of catalysts. The average molecular weight number of PLU, determined by gel permeation chromatography (PL-EMD-950 apparatus equipped with evaporative mass detector, Polymer Laboratories GmbH, Darmstadt, Germany) was of 58000 Da. Calibration was performed on monodisperse polystyrene standard samples with narrow polydispersity (Polymer Laboratories GmbH, Darmstadt, Germany). The schematic structure of the resulted PLU is illustrated in Figure 1.

Blend preparation

Solutions of polyurethane and lignin in dimethylformamide (DMF) were prepared separately, as well as proper volumes mixed to obtain blends with a lignin content of 4.2% (w/w), which was previously found to be optimum for enhancing the polyurethane properties.[13] The resulted light brown liquid, as well as the pure PLU solutions in DMF were cast

at room temperature onto degreased glass plates (220 × 240 mm), by using a knife gap system with a 0.6 mm slit. After casting, the glass plates where immersed for 20 min into a water bath and thermostated at 30 °C, to allow gelation and film detachment. The obtained films were washed thoroughly with distilled water, air-dried in the oven at 60 °C for 24 h and under vacuum (2 mm Hg) at room temperature for another 48 h.

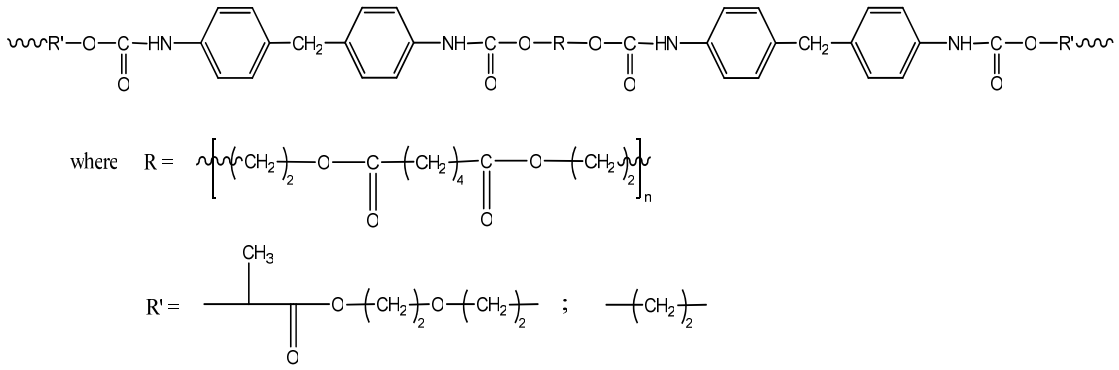

Figure 1: PLU structure

Enzymatic degradation

The PLU and LPLU cast films were cut into 1 cm x 5 cm strips with a thickness of approximately 0.6 mm and immersed in individual glass vials containing 10 mL of an enzyme solution. The peroxidase solution (50 µg/mL; 0.5 POXU/mL), prepared in 200 mM phosphate buffer (pH 7.0), also contains 5 mM H_2O_2. As specified by the provider, one POXU unit represents the amount of enzyme that catalyzes the conversion of 1 µmol H_2O_2 per min, in a system where 2, 2"-azinobis[3-ethylbenzothiazoline-6-sulfonate] (ABTS) is oxidized. The laccase solution (50 µg/mL; 0.05 LAMU/mL) was prepared in 200 mM acetate buffer (pH 5.5), where one LAMU unit is the amount of enzyme which oxidizes 1 mmol syringaldazine per min.

The glass vials were placed in a constant temperature water bath and incubated at 30 °C for 68 h, with short and gentle shaking 4 times per day. The incubation time was established as a compromise between the decay of enzyme activity and the time needed for the occurrence of measurable changes in the thermal degradation and mechanical behavior of the samples. Temperature and pH conditions were set up considering the optimum enzymatic activity. After incubation, all film samples were rinsed in doubly distilled water, washed with ethanol (70% v/v), vacuum-dried at room temperature, and stored at dark in a desiccator until further analysis.

Characterization

Fourier transform infrared spectra were collected by the attenuated total reflectance method (ATR-FTIR), with a DIGILAB Scimitar Series spectrometer equipped with solid cell accessories and a ZnSe crystal with a refraction index of 2.4. Spectra were recorded over the 600-4000 cm^{-1} domain, at room temperature and a resolution of 4 cm^{-1}.

Thermogravimetric (TG) and derivative thermogravimetry (DTG) measurements were performed on a MOM-Budapest Q-1500 D derivatograph. Samples of 50 mg were heated in the air, at a rate of 12 °C/min, with α-Al_2O_3 used as a reference material. A second run was done for each sample. The thermal characteristics determined for each decomposition stage include: temperature corresponding to a mass loss of 10%, respectively 50% ($T_{10\%}$, $T_{50\%}$), temperature corresponding to the maximum mass loss (Tm), onset and offset temperature (T_i, T_f), and mass loss (Wm). The determination errors were of ±2 °C and ±1% mass loss. Kinetic parameters, such as activation energy (Ea) and reaction order (n), were obtained with a minimum confidence degree of 0.95 (C), using the Coats-Redfern method and the Versatile 1.0 software.

Cytotoxicity analysis was performed with extracts of materials, according to ISO 10993-5/1999. The samples were cut into pieces (5 x 1 cm) and separately sterilized by autoclaving at 121 °C for 15 min, respectively, by dipping in 70% ethanol for 2 h. For extraction, the polymer strips were dipped into a 5 mL culture medium containing 5% FCS (fetal calf serum) and incubated for 24 h at 37 °C, in a shaking water bath. The extract was added onto a 3T3 fibroblast cell line culture medium with an initial seeding density of 10^5 cells/well. In the control sample, only the culture medium was added to the cells,

without any extract. After 48 h of incubation at 37 °C, the cytotoxicity of extracts was quantitatively determined by the non-radioactive MTS proliferation assay, based on cellular conversion of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt) in a product soluble in the culture medium, which can be quantified by reading its absorbance at 490 nm, and which is directly proportional with the number of living cells.[21] Before absorbance readings, the fibroblasts had been incubated with MTS for 2 h. The relative percentage of living cells was calculated with respect to the control cells, without any extract.

Tensile measurements were carried out with a TIRATEST 2161 Model Universal Testing machine equipped with a data acquisition module, at room temperature and 20 mm/min cross-head speed. The specimens for tensile testing were dumbbell-cut from both treated and untreated films. A 40 mm benchmark and the original cross-sectional area were used to calculate their tensile properties. Three identical specimens were tested and the results averaged.

RESULTS AND DISCUSSION

ATR-FTIR analysis

The interaction ways among components have a considerable impact on the polymer blend properties and are commonly monitored by infrared spectroscopy techniques. In particular, the spectra obtained by the ATR-FTIR method show the significant infrared signatures of the external material layers to a depth of about 2-3 microns, and represent a powerful tool for investigating the changes occurrring in surface characteristics (*i.e*. composition, structural organization, physical interactions) after blending and enzymatic degradation. For a better comparison, the spectra of LPLU blends and of their pure components, PLU and flax lignin, are presented in absorbance arbitrary units (a.u.) and baseline corrected (Fig. 2). Also, it must be noted that infrared bands frequently result from more than one vibration source, and that any band attribution takes into account the major corresponding contribution.

In the case of flax lignin used for blending, the infrared spectrum is dominated by the very intense peaks from 2916 and 2848 cm^{-1}, attributed to C-H stretching of the aliphatic chains, whereas the corresponding C-H bending vibrations rise at 1471, 1463 and 1430 cm^{-1}. In the C=O stretching region, the free and hydrogen bonded carbonyl groups appear at 1737 and 1702 cm^{-1}, respectively. Numerous weak peaks and bands form a complex pattern between 1314 and 944 cm^{-1}, as a consequence of the overlapped contributions of various stretching and bending vibrations of the C-O bonds from the carboxyl, ester and ether groups, and also of wagging of different C-H bonds.[22] The two weak and broad bands centered at approximately 3400 and 2660 cm^{-1} represent the contributions of the O-H stretching vibrations from the hydroxyl and, respectively, carboxyl groups, whereas the very weak signals from 1594 and 1503 cm^{-1} correspond to the stretching of the aromatic rings.

PLU infrared absorbancies involve different and frequently overlapping contributions from its constitutive aromatic rings, urethane, ester, ether, methyl and methylene groups.[23-25] The C=C stretching vibrations from the MDI rings are the main source of distinct and specific peaks from 1599 and 1413 cm^{-1}, while aromatic C-H twisting appears at 1022 cm^{-1} (in plane; shoulder) and 818 cm^{-1} (out of plane). Characteristic urethane signals occurred at 3325 cm^{-1} (N-H stretching; hydrogen bonded), 1712 cm^{-1} (C=O stretching), 1533 cm^{-1} (C-N stretching coupled with N-H bending and C=O stretching), 1311 and 1225 cm^{-1} (C-N stretching coupled with N-H bending and C-O stretching), and 771 cm^{-1} (N-H wagging). In addition, the free N-H, together with the stretching of the terminal O-H bonds form a broad shoulder centered at 3447 cm^{-1}. The free carbonyls from the ester groups are responsible for the overlapped band from 1732 cm^{-1}, while the hydrogen bonded ones are masked by the signal of urethane carbonyls from 1712 cm^{-1}. Two strongly overlapped bands observed at 1169 and 1142 cm^{-1} are attributable to the symmetrical C(O)-O stretching of the ester groups from PEA and DEGL – as major vibration sources. The unsymmetrical C(O)-O stretching is masked by the 1225 cm^{-1} urethane band. Furthermore, the band at 1067 cm^{-1} is given by the stretching of C-C-O from the polyurethane soft and hard segments, and of C-O-C from DEGL moieties. Methylene asymmetrical and symmetrical stretching determines the peaks at 2922 and 2853 cm^{-1}, while the bending and wagging vibrations are the major contributors for the signals observed at 1458 and 1381 cm^{-1}.

Although blending was made with small lignin amounts (4.2% by mass), a significant increase in the 2922 and 2853 cm^{-1} peak intensities was expected, because of the very strong absorbancies showed by its aliphatic C-H stretching vibrations. However, the data obtained indicate only a very weak increase of these intensities, due to chain reorientation and partial losing of lignin from the surface. Lignin may migrate in depth, due to hydrophobic/hydrophilic interactions, and may be carried out, to a certain extent, by the solvent during gelation in water. Anyway, the presence of lignin in the external layers is confirmed by the slight increase of absorbance values from the 3600-3400 and 1670-1640 cm^{-1} regions, related to the hydrogen bonded carboxyl groups. Also, the free carbonyl band slightly decreases and migrates to 1728 cm^{-1}, whereas the one corresponding to hydrogen bonded carbonyls highly increases and migrates to 1707 cm^{-1}. These results indicate not only the presence of lignin carbonyls and a potential increase in the surface concentration of urethane groups, but also the formation of new and stronger hydrogen bonds. Moreover, all the other bands that could be involved in hydrogen bond formation shift 1-4 cm^{-1} to lower wavenumbers (3323, 1530, 1310, 1221, 1167, 1138, 1065, and 768 cm^{-1}), which supports lignin participation in these physical interactions, the result being a better cohesion and structural organization at surface level. Furthermore, the intensities of bands centered at 1067 and 1138 cm^{-1} increase much more than those at 1221 and 1167 cm^{-1}. These observations, corroborated with the reduction in the intensities of bands related to methylene bending and wagging vibrations, clearly indicate that blend surface contains low lignin amounts, presents fewer MDI/EG and much fewer MDI/PEA chain segments, and is highly enriched in MDI/DEGL structures.

As a consequence, lignin addition does not change only the composition of the external polymer layers, but also actively modifies their entire organization. Since enzymatic degradation under the action of fungal peroxidase and laccase highly depends on surface composition and architecture, there results that lignin addition should substantially transform the stability of LPLU blends, compared to that of PLU. The comparative analysis of the ATR-FTIR spectra of PLU (Fig. 3) and LPLU (Fig. 4) degradation confirms this hypothesis, and also shows major differences between the effects of peroxidase and laccase on both blended and non-blended polyurethanes, as due to their different oxidative mechanisms. For the sake of comparison, all spectra were baseline corrected and normalized to the peak height of the 1413 cm^{-1} absorbance band, which is intensely sufficient and relatively stable at both lignin addition and enzymatic degradation.[26-27]

The effects of peroxidase treatment on the external layers of non-blended polyurethane are important, and seem to be related mainly to the degradation of PEA soft segments, as shown by the comparison of the corresponding infrared spectra (Fig. 3). Thus, the breaking of the ester groups is indicated by the powerful increase of absorbancies at approximately 3600-3250 cm^{-1}, the characteristic region for free and associated N-H and O-H stretching, together with the formation of a well-delimited band at 1670-1630 cm^{-1}, attributable to the hydrogen bonded carbonyls from the carboxylic acids. This process is further confirmed by the lower intensities of C(O)-O stretching (1167 and 1140 cm^{-1}) and aliphatic C-H vibrations. Also, the overlapped carbonyl bands became more distinct, with the wavelength of free ester C=O shifting to a higher value (1734 cm^{-1}), and that of bonded C=O from both ester and urethane groups shifting downwards (1709 cm^{-1}) and rising. On the other hand, the peaks with more significant DEGL and EG contributions (1140 and 1067 cm^{-1}, respectively 1458, 1225 and 1067 cm^{-1}) were less affected than those more characteristic of PEA (1381 and 1167 cm^{-1}). Moreover, the intensity of the 1067 cm^{-1} band actually increases.

In the presence of lignin, surface enrichment in lactate segments permits the oxidative degradation of both PEA and DEGL segments, while the crystalline MDI/EG structures look stable (Fig. 4). Such an assertion is supported by the fact that ester and ether stretching vibrations (1169, 1138 and 1065 cm^{-1}) decrease, while the free carbonyl and all urethane bands remain virtually unchanged. Also, although the aliphatic C-H stretching peaks are more affected by peroxidase treatment in the case of LPLU, methylene bending and wagging vibrations (1458 and 1381 cm^{-1}) are less

reduced, suggesting that lignin and lactate segments were the main enzymatic target. A major difference in the LPLU and PLU degradative ATR-FTIR patterns consists in the almost complete absence of hydroxyl and carboxyl groups in the peroxidase-treated blend. Such flattening of the 3600-3400 cm^{-1} region strongly suggests both degradation of the lignin macromolecules from the blend surface and involvement of the functional groups, newly formed through PEA or DEGL chain scission, in subsequent coupling reactions. Since the carbonyl bands are not reduced, the occurrence of decarboxylation reactions is very unlikely for peroxidase degraded PLU and LPLU.

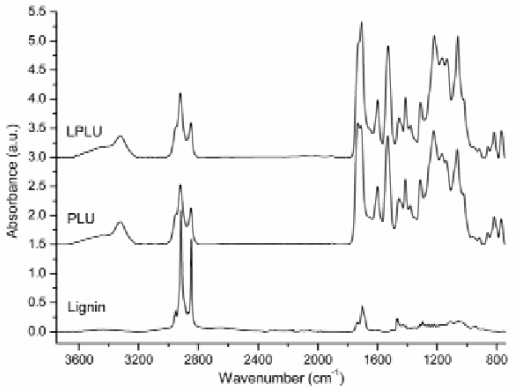

Figure 2: ATR-FTIR spectra of flax lignin, PLU and LPLU

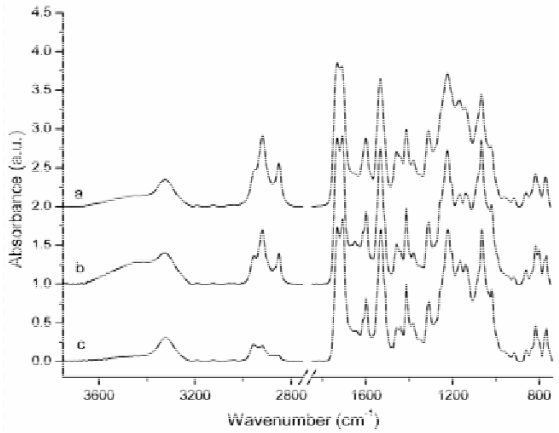

Figure 3: Comparative ATR-FTIR spectra of PLU (a) and enzyme-treated PLU (b – with peroxidase; c – with laccase)

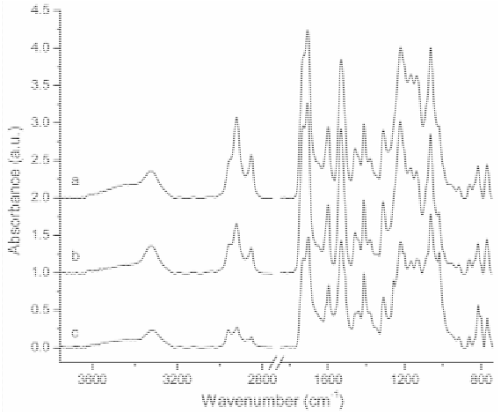

Figure 4: Comparative ATR-FTIR spectra of LPLU (a) and enzyme-treated LPLU (b – with peroxidase; c – with laccase)

The PLU treatment with laccase strongly reduces the C-H vibrations of the aliphatic chains, and moderately lowers the intensities of the free and hydrogen-bonded N-H (Fig. 3). The absorbance intensity of the free carbonyls (1732 cm^{-1}) is also reduced, while that of hydrogen bonded carbonyls (1707 cm^{-1}) remains unchanged. In the ester region, only the peak at 1165 cm^{-1} is slightly reduced, whereas the ether-type peak from 1066 cm^{-1} increases. All these data suggest that laccase oxidizes the PLU aliphatic chains and may use some of the urethane N-H groups for crosslinking processes, and also as chemical mediators. The scission of the ester groups seems to be very limited, yet the elimination of the oxidized aliphatic chains should occur. These processes also take place in the presence of lignin, even if to a lower extent (Fig. 4). However, the significantly reduced intensity of all urethane characteristic absorbancies indicates, beyond any doubt, that lignin addition strongly increases their susceptibility to laccase oxidative degradation. Also, the overall lower intensities showed by laccase-treated LPLU suggest that blend degradation is

accompanied by the formation of a highly porous surface.

It can be assumed that LPLU blends are more stable than PLU to the action of peroxidase, but this stability appears partially compromised both by the sensitivity of lactate segments to degradation and by the extent of lignin migration from the surface during water gelation. Unlike peroxidase, the degradative activity of laccase appears enhanced after blending.

Thermal decomposition

TG/DTG analysis of the samples (Figs. 5-8) was carried out to establish the influence of both lignin addition and enzymatic degradation of the external polymer layers on the bulk stability of biodegradable polyurethanes containing lactate segments in thermo-oxidative conditions.

As generally known, polyurethanes show limited thermal stability, mainly because of their urethane bonds.[28] The main decomposition process, usually occurring between 220 and 400 °C, influenced by the structure of the hard and soft segments, involves two distinct stages.[29] The former one is related to hard segment degradation (scission of urethane bonds, reformation of isocyanate and isocyanate-generated secondary reactions – such as crosslinking, dimerization and trimerization), whereas the soft segments participate to the latter stage together with some of the previously formed secondary products (urea decomposition, oxidation of degraded MDI to benzophenone derivatives through radical intermediates).

As reported elsewhere,[13] the thermal degradation of flax lignin begins at about 150 °C and continues, at quite similar rates, up to more than 550 °C. Decomposition starts with the scission of the α- and β-aryl-alkyl-ether bonds, the dehydration and decarboxylation reactions, followed by the splitting of the aliphatic side chains from the aromatic rings and the breaking of the carbon-carbon linkages between lignin structural units. Since lignin concentration in blends is very low (4.2%) and its decomposition occurs with small fluctuations and at moderate rates, between 200 and 550 °C, the mass loss of blends will be discussed exclusively as a result of polyurethane decomposition, neglecting lignin contribution.

The examination of the DTG diagrams reveals the importance of both lignin addition and enzyme used in preliminary surface oxidative degradation on the PLU behavior, during thermo-oxidative decomposition. Thus, the LPLU blend presents a less extended main decomposition region, without major changes in the degradative pattern, as compared to PLU.

The onset of the first decomposition stage is only 10 °C lower for LPLU and the narrowest, whereas the second stage translates with about 50 °C to lower temperatures. It can be therefore assumed that the presence of lignin inhibits the decomposition of the hard segments and favors the crosslinking reactions, but the radical species formed in the first stage generates chain reactions that reduce the onset of the second stage. Another effect of lignin presence consists in the significant reduction of the mass loss values at the end of the main decomposition region.

All blends have retained up to an about twice higher mass and showed a $T_{50\%}$ up to 67 °C higher than the corresponding untreated and enzyme-treated PLU samples, suggesting that lignin has efficiently participated at numerous crosslinking and condensing reactions.

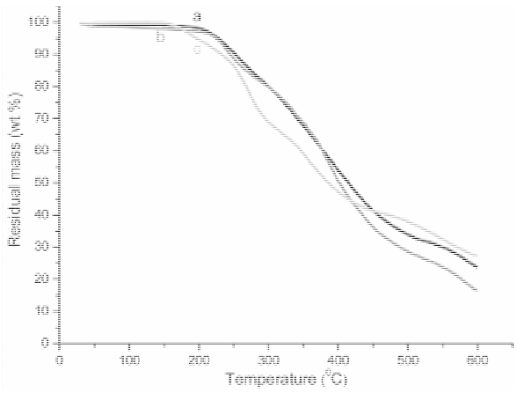

Figure 5: TG curves of initial PLU films (a), and

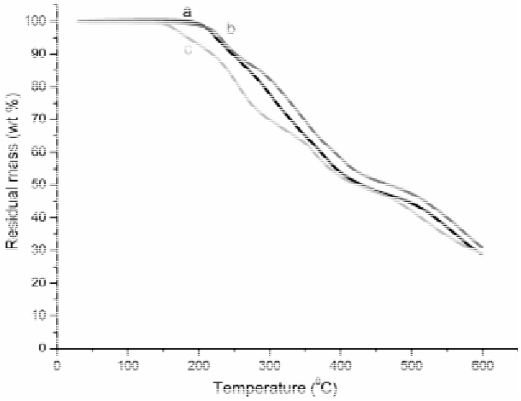

Figure 6: TG curves of initial LPLU films (a), and

after peroxidase (b) and laccase (c) treatments

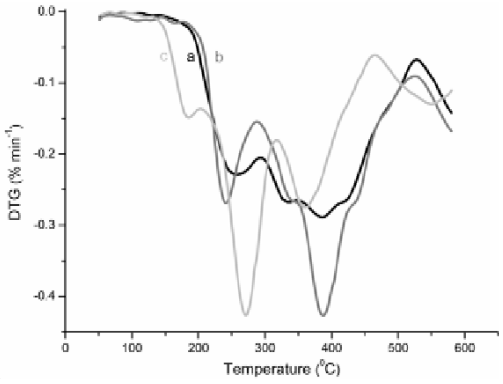

Figure 7: DTG curves of initial PLU films (a), and after peroxidase (b) and laccase (c) treatments

after peroxidase (b) and laccase (c) treatments

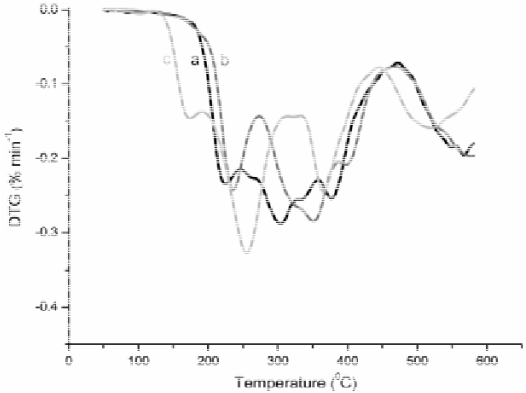

Figure 8: DTG curves of initial LPLU films (a), and after peroxidase (b) and laccase (c) treatments

The values obtained from the TG/DTG analysis for characteristic decomposition temperatures, mass loss, activation energy and reaction order, are presented in Table 1.

Preliminary enzymatic bioerosion generally determines the intensification and more distinct separation of the degradative steps from the main decomposition region, acting like a catalyst or activator of thermal oxidative degradation. However, the peroxidase-treated samples behave in a markedly different way than the laccase-treated ones. Thus, peroxidase pre-treatment accelerates the first decomposition stage of PLU and lowers the Tm from 259 to 240 °C, maintaining the same mass loss amount, onset and offset temperatures. Furthermore, the mass loss grows with about 4.7% and its rate is highly increased in the second stage, for almost similar Tm, T_i and T_f temperatures. These observations correlate well with the scission of the PEA segments and with the enhanced presence of the carboxyl and hydroxyl groups at the polymer surface, as shown by the infrared analysis. In the case of the peroxidase-treated blends, the thermal decomposition pattern is substantially changed and appears somewhat more similar to that of untreated PLU, indicating that peroxidase degraded the lignin from the surface, but its attack on polyurethane soft segments was strongly inhibited.

If peroxidase-treated PLU maintains approximately the same decomposition stages and major differences appear in the mass loss rates, the laccase pre-treatment changes the entire main decomposition region. The onset of decomposition stages is lowered with 45 °C and, respectively, 80 °C, with a second stage composed of two highly distinct steps and Tm values even more intensely reduced (Table 1). This very early and accelerated decomposition should be the result of a previous efficient enzymatic oxidation of the PLU aliphatic chains and of the involvement of urethane groups as laccase mediators. Infrared spectra have indicated that these processes also take place in the presence of lignin, but to a lower extent, which is additionally sustained by the thermal decomposition behavior of LPLU. The thermal degradation profiles confirm the decay of PLU and especially of the lignin structures after enzymatic oxidative treatment, accompanied by an increase in their reactive species forming potential. This could furthermore explain the better separation in distinct decomposition steps for peroxidase and laccase-treated polymers.

The thermal stability of polymers is an important factor, from both processing and applicative perspectives. In particular, many applications demand a preliminary sterilization. On the other hand, the sterilization procedures may provide useful indirect hints on the stability of polymers by evaluating their cytotoxicity before and after such treatments. Polyurethane cytotoxicity is mainly given by the elimination of MDI under its diamine form, following the degradation of the urethane groups. Glycols, lactate and/or adipate low molecular mass compounds, which form the following hard and soft segment degradation, may generate either cytotoxic reactions (but much lower than urethane residues), or even sustain the viability of cells. The cytotoxicity of polymer extracts was tested on fibroblasts, after a preliminary sterilization by

autoclaving at 121 °C for 15 min, respectively by dipping in ethanol 70% for 2 h (Table 2).

Ethanol treatment is a mild procedure frequently used for polyurethane sterilization, while autoclaving, which combines temperature with the superheated water vapors, is very aggressive and usually degrades at slow rates this type of polymers. After ethanol sterilization, cell viability was found as increasing for all blends and all enzymatic pre-treated samples, compared to pure PLU. These results confirm that peroxidase and laccase act especially on PEA and DEGL, and do not provoke the scission of the urethane bonds. Also, the low molecular mass products resulted from PEA and DEGL degradation and extracted in the cell culture media seem to promote cell viability. The positive influence of lignin addition on fibroblasts is exerted to a lower extent than the enzymatic treatment. There are two possible explanations for this favorable lignin effect: its extraction in the cell medium and a better organization of the PLU macromolecular architecture, which leads to lower amounts of toxic extractible compounds. Anyway, the resistance of pure, untreated PLU appears to be much higher in the case of autoclaving than that of all other samples.

Table 1
Thermal decomposition data for studied samples

Sample	$T_{10\%}$	$T_{50\%}$	1st Stage						2nd Stage					
			Tm	T_i-T_f	Wm	Ea	N	C	Tm	T_i-T_f	Wm	Ea	n	C
PLU	252	415	259	191-278	14.20	159.3	1.80	0.9837	386	297-464	41.89	105.1	1.60	0.9729
PLU per.	245	401	240	191-278	14.13	175.1	1.70	0.9937	388	299-459	45.61	117.1	1.55	0.9794
PLU lac.	235	388	178	146-199	4.98	214.7	1.80	0.9883	272	217-419	48.08	77.4	1.75	0.9641
LPLU	248	431	225	181-235	7.67	238.0	1.65	0.9903	304	249-409	37.23	89.8	1.55	0.9713
LPLU per.	252	468	233	176-264	11.46	144.1	1.35	0.9897	352	283-432	32.54	115.7	1.60	0.9740
LPLU lac.	219	423	176	136-189	5.31	204.3	1.75	0.9939	255	200-415	42.14	64.1	1.70	0.9544

per. – peroxidase-treated; lac. – laccase-treated; $T_{10\%}$, $T_{50\%}$ – temperature corresponding to a mass loss of 10%, respectively 50% (°C); Tm – temperature corresponding to the maximum rate of mass loss (°C); T_i, T_f – onset and offset stage temperatures (°C); Wm – mass loss (%); Ea – apparent activation energy (kJ/mol); n – reaction order; C – confidence degree

Table 2
Cell viability of fibroblasts in polymer extracts

Sample	Cell viability after sterilization (%)	
	Ethanol (70%, 2 h)	Autoclaving (121 °C, 15 min)
PLU	78.4	74.1
PLU per.	97.1	55.3
PLU lac.	92.1	60.2
LPLU	86.3	56.5
LPLU per.	96.9	61.8
LPLU lac.	103.2	65.0

per. – peroxidase-treated; lac. – laccase-treated

Mechanical properties

The stress/strain diagrams (Figs. 9-10) confirm the results previously obtained by ATR-FTIR and thermal degradation analyses.

The LPLU blends show a tensile strength more than 3 times higher and a modulus of elasticity more than 4 times higher than PLU, and comparable elongation-at-break and resilience values (Table 3). Consequently, it can be assumed that lignin enhances microphase separation of the segmented PLU, which in turn improves its mechanical properties. Furthermore, the rigid, three-

dimensional lignin macromolecules present numerous active groups in processes of hydrogen bond formation, so that it could interact with the ester and urethane groups from the soft phase and with the nearby hard segments, as well. A supramolecular architecture thus probably results, with lignin incorporated within the flexible polyester chains, and linked through hydrogen bondings with the urethane hard segments, thus increasing the volume and reinforcing the soft phase. However, given its structure, lignin could not add elasticity improvements by itself.

The peroxidase treatment slightly lowers the tensile strength of blends and amplifies the elasticity modulus by 1.7 times, while reducing elongation-at-break. This strong increase of modulus, at the expense of elongation, suggests that soft segment degradation is accompanied by numerous crosslinking reactions. Laccase treatment drastically reduces elongation-at-break and tensile strength of the blends, while the modulus of elasticity remains high, also indicating the occurrence of both crosslinking and soft segment scission. However, oxidative degradations are the preponderant reactions in this last case.

Table 3
Representative mechanical properties for studied samples

Sample	Modulus of elasticity (MPa)	Resilience (MPa)	Tensile strength (MPa)	Elongation at break (%)
PLU	9.82	$11.87 \cdot 10^{-3}$	1.61	79.65
PLU per.	10.33	$6.22 \cdot 10^{-3}$	1.49	60.75
PLU lac.	19.99	$15.59 \cdot 10^{-3}$	1.87	44.27
LPLU	42.24	$15.14 \cdot 10^{-3}$	5.25	80.10
LPLU per.	72.46	$6.98 \cdot 10^{-3}$	5.04	54.75
LPLU lac.	55.70	$7.06 \cdot 10^{-3}$	2.61	28.05

per. – peroxidase-treated; lac. – laccase-treated

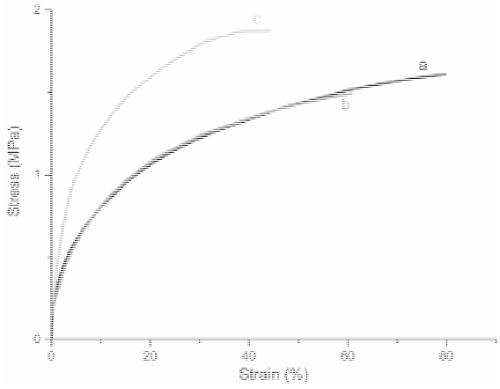

Figure 9: Stress-strain curves for PLU films: initial (a); after treatments with peroxidase (b) and laccase (c)

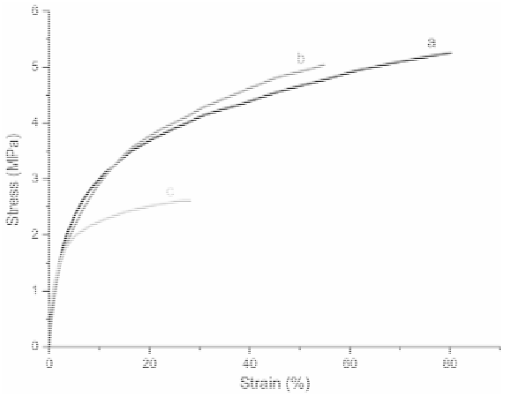

Figure 10: Stress-strain curves for LPLU films: initial (a); after treatments with peroxidase (b) and laccase (c)

CONCLUSIONS

The present study demonstrates the high impact of polyurethane blending with small amounts of lignin (4.3%) on both bulk and surface properties of the resulting material. Lignin interacts with and organizes better the supramolecular structure of polyurethanes containing lactate segments, which in turn improves the stability and the mechanical properties of the blends. Although peroxidase degrades both PLU and lignin, their blends are more stable to enzymatic degradation. On the other hand, laccase activity appears to be enhanced after blending. The presence of lignin significantly reduces the mass loss at the end of the main decomposition region of polyurethanes, increases cell viability after ethanol sterilization and strongly raises the tensile strength and elasticity modulus of the blends.

Despite the need for further investigations, our results clearly show the potential use of low lignin amounts (less than 10%) for increasing mechanical strength, and for delaying and controlling the decomposition of biodegradable polyurethanes in oxidative environments.

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