Open Archive Toulouse Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible.

This is an author’s version published in: http://oatao.univ-toulouse.fr/20320

Official URL: https://doi.org/10.1016/j.indcrop.2014.03.039

To cite this version:
Cachet, Nadja and Camy, Séverine and Benjelloun-Mlayah, Bouchra and Condoret, Jean-Stephane and Delmas, Michel. Esterification of organosolv lignin under supercritical conditions. (2014) Industrial Crops and Products, 58. 287-297. ISSN 0926-6690

Any correspondence concerning this service should be sent to the repository administrator: tech-oatao@listes-diff.inp-toulouse.fr
Esterification of organosolv lignin under supercritical conditions

Nadja Cachet\(^a\), Séverine Camy\(^b\), Bouchra Benjelloun-Mlayah\(^a,\,*\), Jean-Stephane Condoret\(^b\), Michel Delmas\(^a,\,*\)

\(^a\) Compagnie Industrielle de la Matière Végétale (CIMV), 109, Rue Jean Bart, Diapason A, 31674 Labege Cedex, France
\(^b\) Université de Toulouse, INP-Ensiacet, Laboratoire de Génie Chimique (LGC), 4 allée Emile Monso-BP 44362, 31030 Toulouse Cedex 4, France

**ARTICLE INFO**

Keywords:
- Organosolv lignin
- Acetylation
- Supercritical CO\(_2\)
- Quantitative \(^{31}\)P NMR
- Differential Scanning Calorimetry

**ABSTRACT**

An organosolv lignin, extracted in organic acid media, named Biolignin\(^TM\), was acetylated with acetic anhydride in supercritical carbon dioxide (scCO\(_2\)). The effect of moisture and specific surface of lignin sample, temperature (50, 80, 100, 150 °C), reaction time and the use of a catalyst have been studied using analytical techniques such as FT-IR, quantitative \(^{31}\)P NMR and Differential Scanning Calorimetry (DSC).

The reaction appeared to be more efficient when a dried organosolv lignin was used (97% Dry Matter) and with a specific surface of 0.645 m\(^2\)/g. The highest degree of substitution of acetylated samples was obtained after 1 h of reaction at a temperature of 100 °C (180 bar) and in the presence of pyridine as a catalyst.

When compared with conventionally acetylated lignin (using a solvent medium), it appeared that supercritical conditions allowed a higher yield of acetylation and a decrease of the glass transition temperature of the lignin.

1. Introduction

Lignin is composed of three main phenylpropanoid units, namely sinapyl (S), guaiacyl (G), and p-hydroxyphenyl (H) unit and clearly is the most abundant substance based on aromatic moieties in nature. The amount of this natural polymer on the earth is estimated at about 3000 Gt (Singh et al., 2005). It is then a very promising substitute to most of petrochemicals. Chemical modification of lignin, such as esterification, could be used to improve its compatibility for a further transformation. Esterified lignin can be used for example to synthesize durable composites (Olsson, 2011), in unsaturated thermostes (Thielemans and Wool, 2005) or also as a biopolymer precursor for carbon fibers (Zhang and Ogale, 2013). In addition, lignin can be esterified (generally acetylated) in order to determine and quantify its functional groups (El Mansouri and Salvado, 2007; Cateto et al., 2008; Delmas et al., 2011). Lignin is generally esterified with a mixture of anhydride and pyridine (1:1, v/v) and the procedure to recover modified lignin is long and tedious. In the present work we have developed an efficient and environmental friendly procedure to acetylate lignin using supercritical carbon dioxide as a solvent (termed as sc-conditions) and acetic anhydride as a reagent with or without the presence of pyridine as the catalyst. The aim of the study was to develop a “green” and user-friendly protocol in order to quickly recover the acetylated lignins suppressing most of the time-consuming separation/purification steps. Indeed, the use of sc-conditions instead of conventional conditions has several advantages. As a result, it promotes the induced polymer and biopolymer transformations these were effected to be easier and complete (Yalpani, 1993); the organic solvents and toxic by-products are eliminated and/or significantly reduced; and finally, the separation and purification of the final product is generally faster and easier (Young et al., 2003).

These advantages of the use of supercritical carbon dioxide as a solvent for biopolymer chemical modifications have already been demonstrated in the case of cellulose oxidation (Camy et al., 2009).

Following acetylation under sc-conditions, the modified lignin could then be used for further analysis or for a further chemical transformation.

The procedure was optimized on a type of lignin obtained from an organosolv process. This lignin, named Biolignin\(^TM\), is obtained from CIMV refining process (Benjelloun-Mlayah et al., 2009; Benjelloun-Mlayah and Delmas, 2011).

The efficiency of the acetylation was evaluated by conventional analytical methods such as Attenuated Total Reflectance system Fourier Transform Infrared (ATR-FT-IR) as well as quantitative \(^{31}\)P NMR spectroscopy. Comparison between the conventional acetylation of the Biolignin\(^TM\) showed that the physical parameters were not the same for both procedures. It appeared that the conventional
media allowed a complete dissolution of the Biolignin™ sample during the reaction; whereas the Biolignin™ sample acetylated in sc-conditions was kept solid state.

The analysis of the acetylated samples by Differential Scanning Calorimetry (DSC) permitted to compare the physical parameters of the samples.

2. Materials and methods

2.1. Materials

The organosolv lignin studied below is named Biolignin™. Biolignin™ was extracted at pilot scale (CIMV, Pomacle, France) from wheat straw with a mixture of acetic acid/formic acid/water (55:30:15, w/w/w) using the CIMV process (Benjelloun-Mlayah et al., 2009; Benjelloun-Mlayah and Delmas, 2011). The lignin content of the Biolignin™ (i.e. Klason lignin content) was about 89.8 ± 1.5%. The molecular weight in number (Mn) and the molecular weight in weight (Mw) of the Biolignin™ sample were evaluated at 889 and 1719 g/mol, respectively.

All chemicals used were of reagent or HPLC grade and were purchased from Panreac (Castellar del Vallés, España). The CO_2 used for the supercritical esterification was provided by Air Liquide with 99.9% purity.

The supercritical experiments were performed in a stainless steel high pressure vessel with an internal working volume of 90 mL (Top Industrie, France) equipped with an ISCO pump (Teledyne ISEO, model 260D) to fill the reactor from the CO_2 tank.

2.2. Biolignin™ acetylation using supercritical carbon dioxide (sc-conditions)

About 1 g of a Biolignin™ sample with a known moisture content was put in an empty tea bag and placed in the 90 mL-scCO_2 reactor. Before pressurization with CO_2, a large excess of acetic anhydride was added in the reactor (about 5 g, i.e. >10 equiv free OH of the Biolignin™ sample) (Fig. 1). For the experiments which required a catalyst, 100 µL of pyridine were added to acetic anhydride.

The scCO_2 reactor was heated until reaching the desired temperature and then the CO_2 was introduced into the reactor thanks to the pump with a low flow rate (4–6 mL/min) to avoid any Biolignin™ powder dispersion until the desired pressure was reached. In this work, the CO_2 is used as a solvent in order to allow the contact between acetic anhydride and solid lignin.

At the end of the defined reaction time, the pressure was slowly released using the release valve (Fig. 1). When the pressure in the reactor reached the atmospheric pressure, it was unsealed and the modified Biolignin™, contained in the tea bag, was recovered and placed in an oven dryer at 50 °C during 48 h.

The temperatures studied in this work were 50, 80, 100 and 150 °C and the pressure range was 100–180 bar. In this work, the different pressures used were selectively chosen from the experimental liquid-fluid diagram of the CO_2–acetic anhydride binary system previously described by Calvo and de Loos (2006) and Muljana et al. (2011) in order to ensure a single-phase system which is necessary for optimal reaction conditions.

2.3. Biolignin™ acetylation using conventional procedures

Acetylation was conducted using acetyl anhydride and pyridine. Conventional acetylations were performed for comparison of experimental results with acetylated Biolignins™ under sc-conditions. Three reference experiments were conducted (Table 1):

- Control 1: The conditions selected in this experiment were these usually selected for the acetylation of lignin used for a further analytical analysis. Hence, 2 ml of pyridine and 2 ml of acetic anhydride were added to 200 mg of Biolignin™ with known moisture content. The sample was stirred at room temperature during 72 h.
- Control 2: 4ml of pyridine and 4ml of acetic anhydride were placed in a 100 mL-flask equipped with a condenser. 400 mg of Biolignin™ were then added. The sample was stirred at 100 °C during 1 h.
- Control 3: 4ml of 1,4-dioxane, 4ml of acetic anhydride and 100 µL of pyridine were then added. The sample was stirred at 100 °C during 1 h.

The reaction was quenched by adding a mixture of methylene chloride and methanol (8:1, v/v, 18 ml for 200 mg of unmodified Biolignin™). Stirring was maintained at room temperature during 30 min. The mixture was then transferred to a funnel and washed...

---

Fig. 1. Scheme of the Biolignin™ acetylation under sc-conditions.
2.4. Infrared spectroscopic analyses

The modified and unmodified Biolignin™ samples were characterized by infrared analyses using an attenuated total reflectance system (ATR) on a PerkinElmer Spectrum 100 Universal ATR-FTIR instrument equipped with a diamond/ZnSe crystal single reflection. A sweep width of 10,000 Hz was observed, a resolution of 4 cm−1, and a number of scans at a resolution of 4 cm−1.

10 mg of dried unmodified or acetylated Biolignin™ were placed on the crystal plate on which a constant pressure of 85 N/mm² was applied. Each spectrum was obtained after eight scans at a resolution of 4 cm−1.

To compare the obtained spectra, each spectrum was normalized with the intensity of the absorbance peak A1510, which was attributed to a characteristic band of the aromatic skeletal vibrations. The normalization and the baseline correction were performed as it was explained by Gilarranz et al. (2001).

2.5. Quantitative 31P NMR

The 31P NMR technique provides quantitative information for various types of hydroxyl groups and is widely applied to isolated lignin samples (Hu et al., 2011). Approximately 30 mg of dried modified or unmodified Biolignin™ were transferred into a 1.5 mL-sample vial and 400 µL of a mixture of freshly distilled pyridine/deuterated chloroform (1:6:1, v/v) were added. The sample vial was flushed with argon gas, sealed and magnetically stirred at room temperature until complete dissolution.

N-Hydroxynaphthalimide and chromium (III) acetylacetonate were used as the internal standard and relaxation agent, respectively. 100 µL of 0.01 mmol/mL of the internal standard and 100 µL of 0.0143 mmol/mL of the relaxation agent in the solvent system above were added to the sample vial. Finally, 100 µL of 2-chloro-1,3,2-dioxaphospholane was added and the mixture was left at room temperature for 20 min with continuous stirring. The prepared sample solution was then transferred into a 5 mm NMR tube and immediately analyzed.

The spectra were acquired using a Bruker Avance 400MHz spectrometer equipped with a 5 mm TBO BB-1H/31P/D Z-GRD Z104586/0001 probe. A sweep width of 10,000 Hz was observed, and spectra were accumulated with time delay of 25 s between pulses. A pulse width causing 90° flip angle was used. Line broadening of 4 Hz was used in processing spectra. The number of scans was set to 128. All chemical shifts reported in this paper are relative to the reaction product of water with the phosphorylating reagent which has been observed to give sharp signal in pyridine/CDCl₃ at 121.1 ppm (Argyropoulos, 1994). Based on the work of Jasiukaityte et al. (2010), the maximum standard deviation was considered to be 2 × 10⁻² mmol/g and the maximum standard error was 1 × 10⁻¹ mmol/g.

2.6. Differential Scanning Calorimetry (DSC)

The Differential Scanning Calorimetry (DSC) is a technique allowing the study of the thermal behavior of a sample. DSC measurements were performed on a Setaram (Caluire, France) DSC131. The analyses were conducted on 5–10 mg of acetylated or unmodified Biolignin™ in alumina pans sealed by a drilled aluminia lid, under nitrogen atmosphere. Prior to running DSC scans, samples were placed in an oven dried at 50 °C during 48 h to avoid any solvent/water artifacts. In order to eliminate the thermal history of the sample, two step scans were conducted. The first step allowed the annealing of the sample (i.e. the suppressing of the thermal history of the sample). The sample was subjected to an initial scan where it was heated in DSC from 30 °C to 145 °C and maintained at 145 °C during 30 min. The sample was then cooled down to 0 °C and maintained at this temperature during 30 min. The second heating run was used to determine the glass transition temperature (Tg) of the sample. The following temperature program was used: heat ramp from 0 °C to 250 °C at 10 °C/min, isothermal state at 250 °C during 10 min and cooling to room temperature at 30 °C/min under air flow. The cooling phases of the samples DSC scans were not recorded.

3. Results and discussions

The procedures tested to acetylate the Biolignin™ samples under sc-conditions were very user-friendly and allowed a quick recovery of the modified Biolignin™ as shown in Fig. 1. At the end of the reaction, the modified Biolignin™ was easily recovered by untying the small tea bag, in which the Biolignin™ was initially put (Fig. 1).

In an effort to optimize the reaction, a variety of variable were investigated: the specific surface (related to the particle size of the sample) and the moisture of the Biolignin™ samples, the time and the temperature of the reaction and the effect of the catalyst.

<table>
<thead>
<tr>
<th>Table 1: Summary of the experiments of acetylation.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 2 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 3 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 4 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 5 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 6 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 7 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 8 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 9 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 10 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 11 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
<tr>
<td>Control 12 0.645 97% 72 Pyridine/acetic anhydride media (1:1)</td>
</tr>
</tbody>
</table>

Specifications for the experiments of acetylation.
3.1. Influence of specific surface and moisture of the Biolignin™

3.1.1. ATR-FTIR spectra

The moisture of the Biolignin™ sample, linked to its swelling properties, and the specific surface of the sample could have an important role on the efficiency of the acetylation reaction. To determine the effect of the moisture on the reaction, two samples of Biolignin™, 90% and 97% Dry Matter (DM), have been acetylated under supercritical conditions, temperature and pressure were set at 50°C and 100 bar, respectively. The modified Biolignin™ samples obtained were characterized after 6 h of reaction.

The ATR-FTIR spectra of the resulting acetylated Biolignins™ indicated that these above conditions allowed a partial acetylation of the Biolignin™ samples. Indeed, a shoulder band at \( \nu \approx 1741 \text{ cm}^{-1} \) was distinguishable. This band could be attributed to the C=O vibration of aliphatic acetyl (Table 2). In the same manner, the presence of a shoulder band was noted at \( \nu \approx 2939 \text{ cm}^{-1} \) and was attributed to the C=O vibration of aromatic acetyl (Table 2). On the other hand, the characteristic broad band attributed to the hydrogen bonded –OH, at \( \nu \approx 3400 \text{ cm}^{-1} \), was still apparent indicating that the acetylation of the Biolignin™ samples was incomplete.

The ATR-FTIR spectra of acetylated Biolignins™ obtained from “dried” (dry matter (DM) 97%, Exp1) and “wet” Biolignin™ (DM 90%, Exp2) were compared (Fig. 2).

Table 2: Main IR bands assignment of the acetylated and initial Biolignin™ samples.

<table>
<thead>
<tr>
<th>Band position (cm(^{-1}))</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3400</td>
<td>O=H stretching of aromatic and aliphatic OH groups</td>
</tr>
<tr>
<td>2939</td>
<td>C=H asymmetric and symmetric vibration of methyl/methylene groups</td>
</tr>
<tr>
<td>2848</td>
<td>C=H asymmetric and symmetric vibration of methyl/methylene groups/C=H stretching in O—CH(_3) groups</td>
</tr>
<tr>
<td>1823</td>
<td>Characteristic band of acetic anhydride</td>
</tr>
<tr>
<td>1741</td>
<td>C=O stretch of aliphatic acetyl groups</td>
</tr>
<tr>
<td>1711</td>
<td>C=O stretch (unconjugated)</td>
</tr>
<tr>
<td>1651</td>
<td>C=O stretch in conjugated p-substituent carbonyl and carboxyl</td>
</tr>
<tr>
<td>1597</td>
<td>Aromatic skeletal vibration and C=O stretch ring</td>
</tr>
<tr>
<td>1510 (ref)</td>
<td>Aromatic skeletal vibration</td>
</tr>
<tr>
<td>1459</td>
<td>( \nu=\text{CH}_2 \text{ deformation, C—H deformation asymmetric in CH}_3\text{ and CH}_2 )</td>
</tr>
<tr>
<td>1423</td>
<td>Aromatic skeletal vibration with C—H in-plane deformation</td>
</tr>
<tr>
<td>1365</td>
<td>( \nu=\text{of aliphatic chain, acetoxy CH}_2\text{ bending} )</td>
</tr>
<tr>
<td>1327</td>
<td>C=O and C=C of syringyl ring (S-units)</td>
</tr>
<tr>
<td>1222</td>
<td>C=O=C of guaiacyl ring (G-units) (phenolic groups)</td>
</tr>
<tr>
<td>1200</td>
<td>C=O=C of aromatic acetyl groups</td>
</tr>
<tr>
<td>1156</td>
<td>Aromatic C–H in plane deformation, typical of G-units</td>
</tr>
<tr>
<td>1121</td>
<td>Aromatic C—H in plane deformation (S-units), characteristic band of acetic anhydride</td>
</tr>
<tr>
<td>995</td>
<td>Characteristic band of acetic anhydride</td>
</tr>
<tr>
<td>886</td>
<td>Characteristic band of acetic anhydride</td>
</tr>
</tbody>
</table>

As shown in Fig. 3, the ratio \( A_{1741}/A_{1510} \) was higher on the spectrum of the acetylated Biolignin™ from a dry Biolignin™ sample than from a wet Biolignin™ sample (1.055 instead of 0.900 for acetylated Biolignins™ from dry (Exp1) and wet (Exp2) Biolignin™, respectively). Indeed, these experiments indicated that the reaction is more efficient when the initial Biolignin™ is dry.

The influence of the specific surface was also studied. The specific surface is defined as the accessible area of a solid surface per unit mass of material. In the case of lignin powder and thanks to a better contact between lignin and the reagent (acetic anhydride), its reactivity could be enhanced by a higher specific surface. To increase the specific surface of the samples, the unmodified Biolignin™ was graded at two different stages:

- A rough grinding (average particle diameter: 1000 µm) which led to a specific surface of 0.335 m²/g
- A medium-fine grinding (average particle diameter: 68 µm) which led to a specific surface of 0.645 m²/g

Thus, the reactivity of a roughly graded sample (Exp1, specific surface of 0.335 m²/g) and the one of a medium-fine grinded sample (Exp3, specific surface of 0.645 m²/g) were compared.

As shown in Fig. 3, the use of dry Biolignin™ with a higher specific surface induced a slightly better acetylation (\( A_{1741}/A_{1510} = 1.060 \) instead of 1.055 and \( A_{1741}/A_{1510} = 1.715 \) instead of 1.651 for, respectively Exp3 and Exp1).

From these results, the following experiments were done using a dry Biolignin™ (DM 97%) with a specific surface of 0.645 m²/g.

3.1.2. Influence of reaction time and temperature

Esterification is known to be an equilibrated and slow reaction. Traditionally, 24 h to few days of reaction are required in conventional media to esterify lignin samples. Thus, after 6 h of reaction under the above sc-conditions, the acetylation of Biomliignin™ samples might not have reached the equilibrium. Few experiments were proceeded to determine the kinetics of reaction: 6, 10 and 24 h of reaction were respectively tested on dry Biomliignin™ samples (97% DM) with a specific surface of 0.645 m²/g (Exp3, 4 and 5, respectively). One of the goals of using supercritical conditions instead of conventional conditions was to acetylate the Biolignin™ in a limited reaction time. Then, the maximum reaction time was set to 24 h.

The ratios \( A_{1741}/A_{1510} \) and \( A_{2000}/A_{1510} \) were measured on FTIR spectra of the recovered acetylated Biolignin™ samples (Fig. 3).
According to these measurements, the acetylated Biolignin™ samples recovered after 6 and 10 h of reaction had a similar degree of substitution (DS) of aliphatic hydroxyl groups ($A_{1741}/A_{1510}$ of 1.060 and 1.076, respectively) and aromatic hydroxyl groups ($A_{1200}/A_{1510}$ of 1.715 and 1.754, respectively). After 24 h of reaction, the acetylated Biolignin™ sample seemed to have a higher DS of aliphatic hydroxyl groups ($A_{1741}/A_{1510}$ of 1.484, Exp5). The shoulder band at $\sim 1200$ cm$^{-1}$, attributed to the C–O vibration of aromatic acetyl groups, was also stronger than on the previous FTIR spectra ($A_{1200}/A_{1510} = 2.044$), indicating a better acetylation of aromatic hydroxyl groups. In the above conditions (50°C, 100 bar), 24 h of reaction allow the acetylation of a Biolignin™ sample with a DS a bit lower but in a same range that to the one obtain in conventional esterification after 72 h of reaction in presence of a large excess of pyridine (Exp Control 1, Fig. 3).

Since temperature is expected to have a positive influence on the esterification reaction, three temperatures, superior to critical temperature of CO$_2$, were tested: 50°C, 80°C and 100°C (Exp5, 6
Duration of these experiments was set at 24 h. Depending on the temperature, the pressure was adapted from 100 bar to 180 bar in order to stay in a single-phase system during the reaction (Table 1).

According to the ratio \( A_{1741}/A_{1510} \) on the obtained FTIR spectra, the DS of aliphatic hydroxyl groups by acetyl groups seemed to increase with the temperature (Fig. 3). It is interesting to note that when the temperature of the reactor was set to 100 °C, the ratio \( A_{1741}/A_{1510} \) was lower than the one of the experiment proceeded at 80 °C (\( A_{1741}/A_{1510} = 1.579 \) and 1.675, respectively). However, the DS of aromatic hydroxyl groups seemed to increase with the temperature. Indeed, the ratio \( A_{1200}/A_{1510} \), was higher on the FTIR spectrum of Exp 7 than on the spectra of the previous experiments. A shoulder band at \( \nu \approx 1760 \text{ cm}^{-1} \) also appeared (not shown), this last band was attributed to the C=O vibration of aromatic acetyl groups. Finally, the intensity of the band assigned to free phenolic groups (\( \nu \approx 1510 \text{ cm}^{-1} \)) decreased (not shown) and the broad band corresponding to the hydrogen bonded –OH (\( \nu \approx 3400 \text{ cm}^{-1} \)) had nearly disappeared on the FTIR spectrum of the Biologin™ acetylated at 100 °C. Then, it seemed that the sum of aliphatic and aromatic acetyl groups increased with the temperature during the reaction.

From these results, the temperature of the reaction was set to 100 °C for the following experiments.

3.1.3. Effect of pyridine as a catalyst

Without any catalyst, the acetylation of the Biologin™ in a sc-CO₂ reactor required 24 h of reaction to obtain a DS of hydroxyl groups close to the one obtained after 72 h of reaction in conventional media (Exp Control 1, acetic anhydride/pyridine 1:1 v/v). The use of a catalyst may considerably reduce this reaction time. As it was used in acetylation of lignin in conventional media, pyridine was chosen as the catalyst of the reaction. Thus, 100 µL of pyridine were added to the acetic anhydride at the beginning of the reaction. The kinetics of the reaction was studied: The experiments 8, 9 and 10 were respectively stopped after 24, 6 and 1 h of reaction (Table 1). The recovered acetylated Biologin™ samples were then analyzed by ATR-FTIR. After 24 h of reaction, it seemed that the catalyst did not allow the recovery of an acetylated Biologin™ sample with a dramatically higher DS of its hydroxyl groups compared to the same experiment without catalyst (Exp 8 compared to Exp 7, Fig. 3). The DS obtained after 1 and 6 h of reaction (Exp 10 and 9, respectively) seemed to be almost identical of the one observed after 24 h of reaction. These values were very similar to those observed on Biologin™ samples acetylated in conventional media (Fig. 3). Thus, with pyridine as a catalyst, the reaction time could be easily reduced to 1 h under the above sc-conditions.

To check the catalytic effect of pyridine, a control experiment (Exp 11) was conducted in the same conditions as Exp 10 (1 h, 100 °C, 180 bars) without pyridine. As shown in Fig. 3, the DS of the acetylated Biologin™ seemed to be lower in absence of pyridine (Exp 11 compared to Exp 10).

The catalytic effect of pyridine for the acetylation of Biologin™ under sc-conditions is then demonstrated.

The DS observed on acetylated Biologin™ samples after 24 h of reaction and under the applied sc-conditions (100 °C, 180 bar), were close whatever pyridine was added or not. Considering that few hydroxyl groups were still free, it is possible that the steric hinderance and/or the conformation of lignin fragments prevent a higher substitution of the hydroxyl groups. Thus, the presence of a catalyst cannot improve the substitution of these hydroxyl groups. This could explain that the DS of Exp 8 (with pyridine) was close to the one observed in Exp 7 (without pyridine).

According to the FTIR analyses, the best supercritical conditions defined by the above experiments were 100 °C, 180 bar, 1 h of reaction in presence of pyridine as a catalyst (100 µL). Fig. 4 gave an overview of the FTIR spectra of Biologin™ before acetylation, sc-acetylated Biologin™ with non-optimized conditions (Exp 3, 6 h, 50 °C; 100 bar, without catalyst) and sc-acetylated Biologin™ with optimized conditions (Exp 10, 1 h, 100 °C; 180 bar, with pyridine as a catalyst).

The FTIR analyses gave a global view of the DS of the acetylated Biologin™ hydroxyl groups. However the lack of accuracy of this kind of analysis did not allow a reliable quantification of the Biologin™ functional groups. To quantify the functional groups, the acetylated Biologin™ samples were analyzed by quantitative 31P NMR.
3.2. Quantification of the functional groups in the acetylated Biolignin™ samples by $^{31}$P NMR

Quantitative $^{31}$P NMR is a method of choice to quantify functional groups in lignins (Pu et al., 2011). Indeed, in addition to the quantification of some functional groups, this technique gives precious information on the distribution of the free phenolic hydroxyls in the three main units of the Biolignin™ (i.e. p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S)-units).

Table 3 summarizes the results of the quantitative $^{31}$P NMR analysis of some phosphitylated Biolignin™ samples before and after acetylation. Before acetylation, the Biolignin™ samples contained 3.14 mmol/g of free hydroxyl groups. The hydroxyl content was in accordance with the one indicated in the literature for...
The GC-FID chromatograms allow the quantification of released acetyl groups which correspond to free hydroxyl groups of the unmodified sample (Mansson, 1983). As the Biolignin™ seemed to be only partially acetylated using conventional procedures, we can wonder whether the quantification by this technique is really effective to assess the whole hydroxyl content.

Focusing on experiments using conventional procedures, it seems that the conditions inducing the higher degree of substitution were those of Control 1 (72 h, room temperature, anhydride/pyridine 1:1, v/v). In this case, the most acetylated Biolignin™ samples were those of Control 1 (72 h, room temperature, anhydride/pyridine 1:1, v/v). In this case, 31P NMR analysis of the three acetylated Biolignin™ samples indicated that DS(Control 1) > DS(Control 2) > DS(Control 3) (89.2%, 82.6% and 78.6%, respectively). These results seem coherent in regard to the quantity of pyridine used. Indeed, pyridine was added in large excess, as solvent and catalyst, in Control 1 and 2 whereas it was added only in catalytic quantity in Control 3. It can be deduced that in solvent media, pyridine plays an active role as a catalyst but also as a solvent.

According to the 31P NMR quantification, the ratio of free phenolic H, G and S-units in the unmodified Biolignin™ is 1/6/3. After acetylation of the Biolignin™ samples, using conventional procedures and in severe conditions, 100.0% of the phenolic hydroxyls from H-units and from 72.9 to 100.0% of the phenolic hydroxyls from S-units were acetylated (Table 3). The phenolic hydroxyls from G-units were the only ones for which all the tested experiments did not allow a complete acetylation. For the experiments conducted in solvent media, the best G–phenolic hydroxyls acetylation was obtained in Control 1, with 92.2% of G-phenolic acetates at the end of the reaction. The best sc-experiment 10 (Exp10, Table 3) slightly improved the degree of substitution until obtaining 95.1% of G-phenolic hydroxyls acetylated at the end of the reaction.

Exp10 and Control 3 experiments shared the same experimental parameters (100 °C, 1 h of reaction, 3 drops of pyridine as catalyst of the reaction), except that Exp10 was performed under supercritical condition whereas Control 3 was conventionally conducted. It is then very interesting to especially focus on these two experiments and compare their results. At the end of the reaction, the DS of hydroxyl groups of the acetylated Biolignin™ in Control 3 was considerably lower than the one in Exp10 (78.5% of hydroxyls acetylated in Control 3 against 92.1% in Exp10). In addition to contain a significant amount of free aliphatic hydroxyls (13.8% of the total aliphatic hydroxyls), only 91.3% of S-phenolic hydroxyls of Control 3 were acetylated, which is the second lowest DS of S-phenolic hydroxyls after the one of Exp4 (Table 3). In the case of Exp10, all the S-phenolic hydroxyls were acetylated.
These results showed that, in addition to being a quicker and a more user-friendly procedure, the reaction under supercritical conditions allowed a better acetylation of the Biolignin™ sample.

In sc-Exp12 (Table 1), the temperature of the reaction was increased to 150 °C in order to check if a higher temperature could improve the DS of the Biolignin™ hydroxyl groups compared to Exp10. It appeared that this increase of the temperature did not allow better acetylation. On the contrary, the modified Biolignin™ of Exp12 seemed to be slightly less acetylated than the one of Exp10 (Table 3). Thus, in supercritical conditions, increasing the temperature up to 100 °C has a reverse effect: instead of improving the DS of hydroxyl groups, it seemed to decrease it.

It is noteworthy that, physically speaking, conventional acetylation of the Biolignin™ was completely different from the one under sc-conditions. Indeed, in conventional procedure, the Biolignin™ sample is insoluble at the beginning. Then, the “modified” Biolignin™ sample becomes gradually soluble in the medium until reaching a complete solubility at the end of the reaction. Finally, after extraction, purification and solvent evaporation, the obtained acetylated Biolignin™ is recovered as a solid powder.
Under sc-conditions, the Biolignin\textsuperscript{TM}/acetylated Biolignin\textsuperscript{TM} samples always remain solid during their chemical transformation. Despite these huge physical differences, similar DS of hydroxyl groups were obtained with the two studied procedures (conventional and sc-conditions), even if those obtained in sc-optimized experiments could be slightly higher. However, according to the procedure proceeded, the acetylated Biolignin\textsuperscript{TM} samples had a different behavior: conventionally acetylated Biolignins\textsuperscript{TM} were soluble in a variety of organic solvents (acetone, 1,4-dioxane, THF, etc.) while sc-acetylated Biolignins\textsuperscript{TM} were only slightly soluble in these solvents. Some physical properties of the acetylated Biolignin\textsuperscript{TM} samples might be impacted by these chemo-physical differences. Then, the thermal properties of each sample were evaluated by Differential Scanning Calorimetry (DSC).

3.3. Thermal properties of the acetylated Biolignin\textsuperscript{TM} samples

The thermal properties of the initial and acetylated Biolignin\textsuperscript{TM} samples were measured by DSC. DSC is the most widely accepted method for determining the glass transition temperature (\(T_g\)) of lignin or modified lignin samples (Glasser, 2000). The \(T_g\) of dry lignin is often difficult to detect due to the complex structure of this polymer. However, it is sometimes possible to detect the range of the change in the curve (Fox and McDonald, 2010). When lignin is subjected to a DSC scan, an endothermic enthalpy relaxation process usually occurs and may affect the \(T_g\) determination measurement. For this reason, it is often recommended to subject the sample to an initial scan (above its \(T_g\)) in order to eliminate the stored thermal history within the polymer amorphous non-equilibrium configuration (Cui et al., 2013). Each sample was thus subjected to an initial DSC scan from 30 °C to 145 °C under nitrogen atmosphere to anneal the polymer. A preliminary scan confirmed that no endothermic or exothermic reactions occurred below 145 °C.

The thermal analysis of the studied samples is shown in Fig. 5. A small endothermic peak at 125 °C, present on all recorded thermograms, is an artifact due to the coefficient of expansion of the Al pans (Al: ~24 ppm/K, DSC sensor: ~9 ppm/K).

A preliminary DSC scan from 30 °C to 400 °C allowed us to identify a large exothermic peak between 220 °C and 280 °C on all DSC profiles (not shown). This large exothermic peak might be attributed to the breaking of the side chains of lignin fragments (Vallejos et al., 2011).

The glass transition region reported in the literature for several types of lignins is between 90 °C and 180 °C (Lisperguer and Perez, 2009). The \(T_g\) of initial Biolignin\textsuperscript{TM} was identified at 110 °C. This value is in accordance with the reported \(T_g\) of organosolv lignins (Sammons et al., 2013). Except for the sample Control 3, the acetylated Biolignins\textsuperscript{TM} exhibited a \(T_g\) lower than the initial Biolignin\textsuperscript{TM} (Fig. 5). This result confirmed previous studies on the decreasing of the glass transition temperature when lignin is esterified (Fox and McDonald, 2010).

In the literature, it is shown that the greater the number of carbon atoms in ester substituents of lignin, the greater the lignin ester \(T_g\) reduction (Ghosh, 1998; Fox and McDonald, 2010). The reduction of glass transition temperature was explained by the increase of the polymer free volume and by the disruption of hydrogen bonds within the lignin polymer (Glasser et al., 1984). Indeed, this leads to increase the mobility within the lignin molecules and hence the reduction of the glass transition. In the same way, Gifford et al. patented the mixing of two lignin esters with a different number of carbon atoms (Gifford et al., 2010). They showed that the increase of the lignin ester with the higher number of carbon atoms led to a decrease of \(T_g\) of the resulting mixed lignin ester. To our knowledge, there is no study on the effect on \(T_g\) related to the DS of hydroxyl groups of lignin esters. However, considering our results, it seems consistent to say that the more a lignin is acetylated, the more the \(T_g\) of the resulting lignin ester is decreased. The measured \(T_g\) illustrated in Fig. 5 were in line with this hypothesis. Indeed, according to the previous results, the evaluation of the DS of hydroxyl groups of Biolignin\textsuperscript{TM} esters indicated that DS(Exp10)>DS(Control 1)>DS(Control 2)>DS(Control 3). Thus, \(T_g\) (Exp10)>\(T_g\) (Control 1)>\(T_g\) (Control 2)>\(T_g\) (Control 3).

The \(T_g\) of Exp10 was determined as 99 °C, which is much lower than the \(T_g\) of the other experiments (between 108 °C and 110 °C). If we consider that the decrease of \(T_g\) is linearly linked with the DS of lignin hydroxyl groups, the DS of Exp10 compared to the one of the other experiments (Table 3) cannot explain such a difference. The phenolic hydroxyl groups of Exp10 were almost completely acetylated (98.0%) while the best conventional acetylation yielded a maximum acetylation of 96.7% for the phenolic hydroxyl groups of the Biolignin\textsuperscript{TM} sample (Control 1). The increase of mobility within the lignin ester molecules is mainly due to the decrease of H-bonds involving phenolic hydroxyl groups (Glasser et al., 1984). Then, the decrease of residual phenolic OH in Exp10 may partially explain the decrease of \(T_g\). However, a deeper study would be needed to confirm this hypothesis. It is also possible that the procedure of acetylation directly affect the \(T_g\) of the acetylated Biolignin\textsuperscript{TM}. Indeed, \(T_g\) is closely related to the presence of H-bonds and the mobility within the lignin. As the sample remained in its solid state during the acetylation under sc-conditions, the H-bond interactions of sc-acetylated Biolignin\textsuperscript{TM} may be lower than the ones of conventionally acetylated Biolignin\textsuperscript{TM}. This could also explain the lower \(T_g\) obtained in Exp10 sample compared to the one of Controls 1, 2 and 3.

4. Conclusion

The acetylation of the organosolv lignin under sc-conditions compared to conventional acetylation presents numerous advantages. Firstly, a considerable reduction of the reaction time has been demonstrated in this work (1 h compared to few days in the most of case). Moreover, the use of pyridine was dramatically reduced. The measurement of free residual hydroxyl groups after acetylation indicated that, under the optimized sc-conditions, 92% of the hydroxyl groups were acetylated against around 89% in conventional media. Thus, the degree of substitution (DS) of hydroxyl groups could be higher under sc-condition media compared to conventional conditions. When a production process is envisaged, a very important advantage arises from the elimination of complex extraction/purification steps. In this case, a very “clean” product can be easily recovered by evaporation of carbon dioxide and unreacted anhydride, when return to atmospheric conditions.

On a physical point of view, the acetylation reaction of the Biolignin\textsuperscript{TM} under sc-condition was different than the one obtained by the conventional procedures where the Biolignin\textsuperscript{TM} sample became gradually soluble in the medium until reaching a complete solubility at the end of the reaction. Under sc-condition, the Biolignin\textsuperscript{TM}/acetylated Biolignin\textsuperscript{TM} samples always remained in their solid state during their chemical transformation. In spite of this huge physical difference during the reaction, the DS of hydroxyl groups of acetylated Biolignin\textsuperscript{TM} seemed to be approximately the same. However the procedure induced a different physical behavior. The conventionally acetylated Biolignin\textsuperscript{TM} samples were soluble in a variety of organic solvent (THF, 1,4-dioxane, acetone, etc.) while the acetylated Biolignin\textsuperscript{TM} samples under sc-condition were poorly soluble in the same solvents. Finally, the study of the thermal behavior of the samples indicated that lignins acetylated under sc-conditions presented a glass transition temperature lower than conventionally acetylated lignins.
Acknowledgements

The authors thank the CIMV Company for providing the wheat straw lignin used in this work. The authors are also grateful to Dr. Pierre Lavedan and Dr. Marc Vedrenne for their precious help in the establishment of the quantitative $^{31}$P NMR procedure.

References


