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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™, 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*-hydroxylphenyl (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 300Gt (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 thermosets (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™, 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™ showed that the physical parameters were not the same for both procedures. It appeared that the conventional

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

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 (M_n) and the molecular weight in weight (M_w) 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 Vallès, 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 Isco, model 260D) to fill the reactor from the CO_2 tank.

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

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 eq/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: 4 mL of pyridine and 4 mL 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: 4 mL of 1,4-dioxane, 4 mL of acetic anhydride and 100 μL of pyridine 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.

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

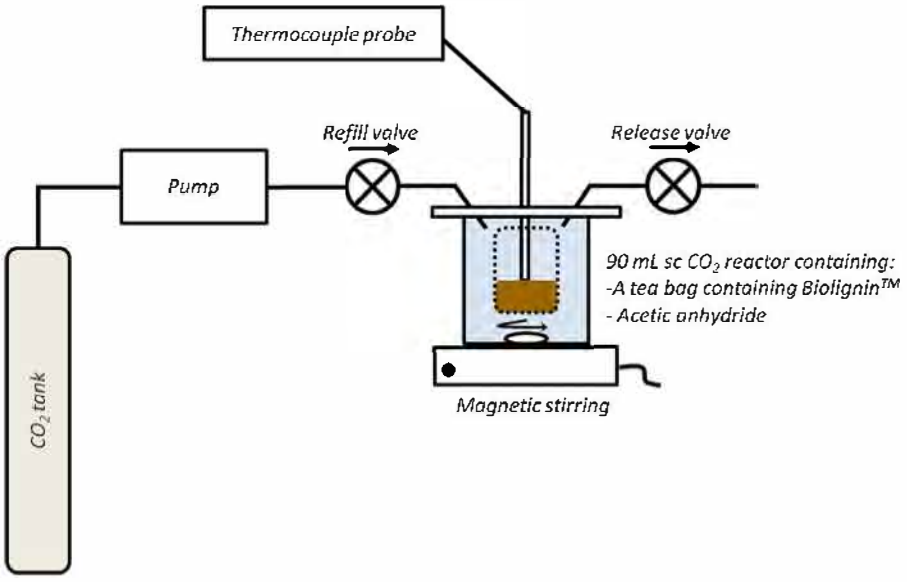

Fig. 1. Scheme of the Biolignin™ acetylation under sc-conditions.

Table 1
Summary of the experiments of acetylation.

	Biolignin™ used		T (°C)	P (bar)	Reaction time (h)	Catalyst
	Specific surface (m^2/g)	Dry matter (DM)				
Control 1	0.645	97%	25	P_{atm}	72	Pyridine/acetic anhydride media (1:1)
Control 2	0.645	97%	100	P_{atm}	1	Pyridine/acetic anhydride media (1:1)
Control 3	0.645	97%	100	P_{atm}	1	1,4-Dioxane/acetic anhydride media (1:1) + pyridine (cat.)
Exp1	0.335	97%	50	100	6	–
Exp2	0.335	90%	50	100	6	–
Exp3	0.645	97%	50	100	6	–
Exp4	0.645	97%	50	100	10	–
Exp5	0.645	97%	50	100	24	–
Exp6	0.645	97%	80	160	24	–
Exp7	0.645	97%	100	180	24	–
Exp8	0.645	97%	100	180	24	Pyridine (cat.)
Exp9	0.645	97%	100	180	6	Pyridine (cat.)
Exp10	0.645	97%	100	180	1	Pyridine (cat.)
Exp11	0.645	97%	100	180	1	–
Exp12	0.645	97%	150	180	1	Pyridine (cat.)

with, respectively, 2 M HCl aqueous solution, $NaHCO_3$ aqueous saturated solution, and distilled water. The organic phase was collected and dried with $MgSO_4$. After removing $MgSO_4$, the solvent was evaporated under reduced pressure. Acetylated Biolignin™ was recovered as a sparkling dark powder. The sample was kept in an oven dryer at 50 °C during 48 h and stored under dry atmosphere.

These reference experiments are termed "acetylation using conventional procedures".

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.

10 mg of dried unmodified or acetylated Biolignin™ were placed on the crystal plate on which a constant pressure of 85 N/mm^2 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 A_{1510}, which was attributed to a characteristic band of the aromatic skeletal vibrations. The normalization and the baseline correction were processed as it was explained by Gilarranz et al. (2001).

2.5. Quantitative ^{31}P NMR

The ^{31}P NMR technique provides quantitative information for various types of hydroxyl groups and is widely applied to isolated lignin samples (Pu 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 distillated 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-Hydroxynaphtalimide and chromium (III) acetylacetanoate 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 400 MHz 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 phosphitylating reagent which has been observed to give sharp signal in pyridine/$CDCl_3$ 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^{-2} 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 alumina 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 (T_g) 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 2
Main IR bands assignment of the acetylated and initial BioligninTM samples.

Band position (cm^{-1})	Assignment
3400	O—H stretching of aromatic and aliphatic OH groups
2939	C—H asymmetric and symmetric vibration of methyl/methylene groups
2848	C—H asymmetric and symmetric vibration of methyl/methylene groups/C—H stretching in O—CH_3 groups
1823	Characteristic band of acetic anhydride
1741	C=O stretch of aliphatic acetyl groups
1711	C=O stretch (unconjugated)
1651	C=O stretch in conjugated p-substituent carbonyl and carboxyl
1597	Aromatic skeletal vibration and C=O stretch ring
1510 (ref)	Aromatic skeletal vibration
1459	O—CH_3 deformation, C—H deformation asymmetric in CH_3 and CH_2
1423	Aromatic skeletal vibration with C—H in-plane deformation
1363	C—H of aliphatic chain, acetoxy CH_3 bending
1327	C—O and C—C of syringyl ring (S-units)
1222	C—O—C of guaiacyl ring (G-units) (phenolic groups)
1200	C—O—C of aromatic acetyl groups
1156	Aromatic C—H in plane deformation, typical of G-units
1121	Aromatic C—H in plane deformation (S-units), characteristic band of acetic anhydride
995	Characteristic band of acetic anhydride
886	Characteristic band of acetic anhydride

3.1. ATR-FTIR spectra

3.1.1. Influence of specific surface and moisture of the Biolignin™

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 $\nu \approx 1741\ cm^{-1}$ was distinguishable. This band could be attributed to the C=O vibration of aliphatic acetyls (Table 2). In the same manner, the presence of a shoulder band was noted at $\nu \approx 1200\ cm^{-1}$ and was attributed to the C—O vibration of aromatic acetyls (Table 2). On the other hand, the characteristic broad band attributed to the hydrogen bonded –OH, at $\nu \approx 3400\ cm^{-1}$, 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).

The characteristic bands of acetic anhydride ($\nu \approx 1823$, 1121, 995 and 896 cm^{-1}) indicated that both acetylated Biolignin™ samples contained traces of unreacted acetic anhydride.

According to the literature the band at $\nu \approx 1510\ cm^{-1}$ is one of the three characteristic bands assigned to aromatic skeletal vibrations (Gilarranz et al., 2001). This band is well defined in every unmodified and modified Biolignins™ spectra. It could thus be used to normalize the spectra (the spectra were normalized with the intensity of the absorbance peak A_{1510} as it was indicated by Gilarranz et al. (2001).

The IR spectra were studied by calculating the ratio of the absorbance of a specific band with the one of the band at $\nu \approx 1510\ cm^{-1}$. Thus, the calculated ratios were used to compare an IR spectrum with each other. As noted above, the bands at $\nu \approx 1741$ and $1200\ cm^{-1}$ could be attributed to the C=O vibration of aliphatic acetyls and the C—O vibration of aromatic acetyls, respectively. Even if no precise value could be determined, the ratios A_{1741}/A_{1510} and A_{1200}/A_{1510} could then give an indication on the degree of substitution (DS) of hydroxyl groups by acetyl groups in the acetylated sample. Indeed, the greater these ratios, the greater the DS.

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 grinded at two different stages:

- A rough grinding (average particle diameter: 1000 μm) which led to a specific surface of 0.335 m^2/g
- A medium-fine grinding (average particle diameter: 68 μm) which led to a specific surface of 0.645 m^2/g

Thus, the reactivity of a roughly grinded sample (Exp1, specific surface of 0.335 m^2/g) and the one of a medium-fine grinded sample (Exp3, specific surface of 0.645 m^2/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 m^2/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 Biolignin™ 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 Biolignin™ samples (97% DM) with a specific surface of 0.645 m^2/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_{1200}/A_{1510} were measured on FTIR spectra of the recovered acetylated Biolignin™ samples (Fig. 3).

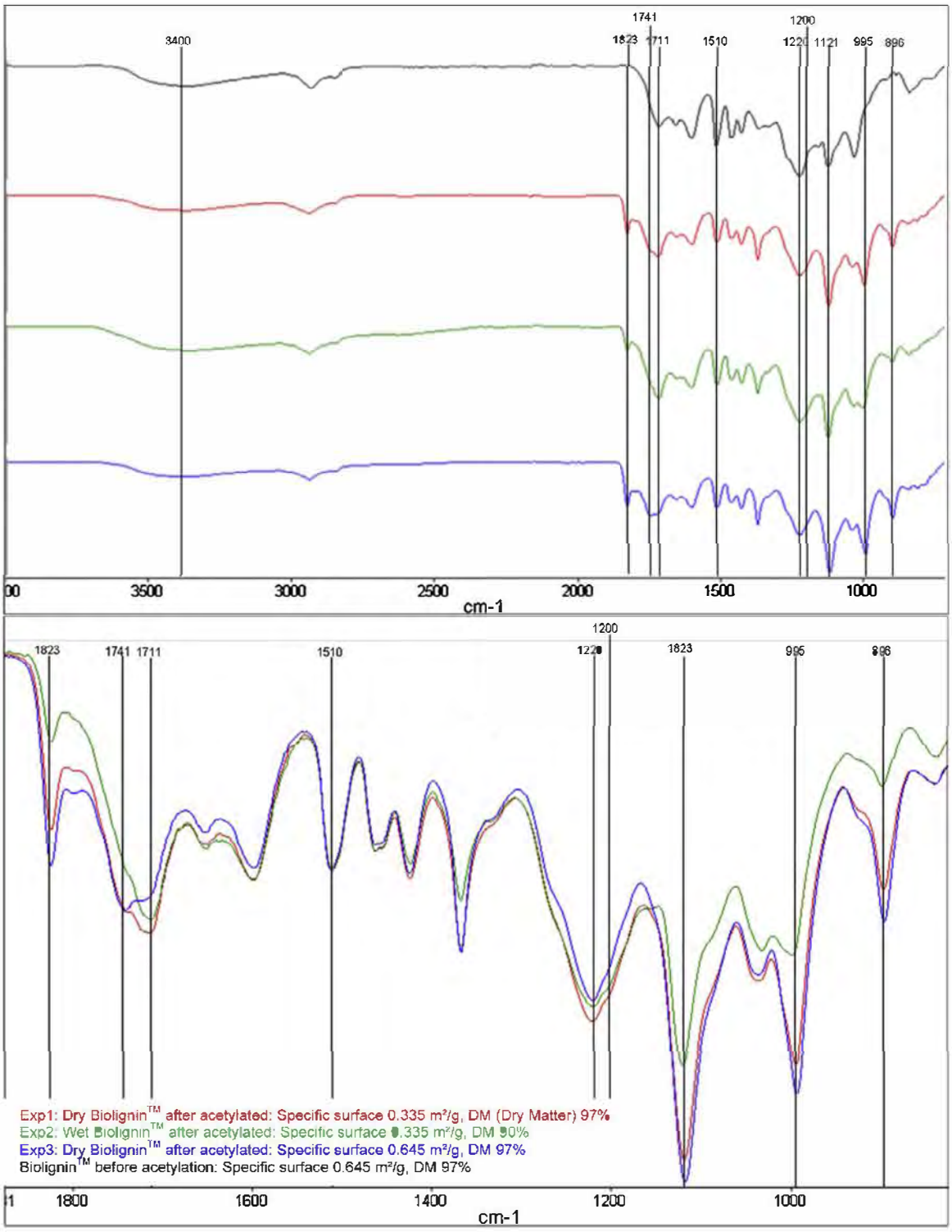

Fig. 2. ATR-FTIR spectra of initial Biolignin™ and acetylated Biolignins™ from Exp1-3.

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 $\nu \approx 1200\,cm^{-1}$, attributed to the C—O vibration of aromatic acetyls, 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

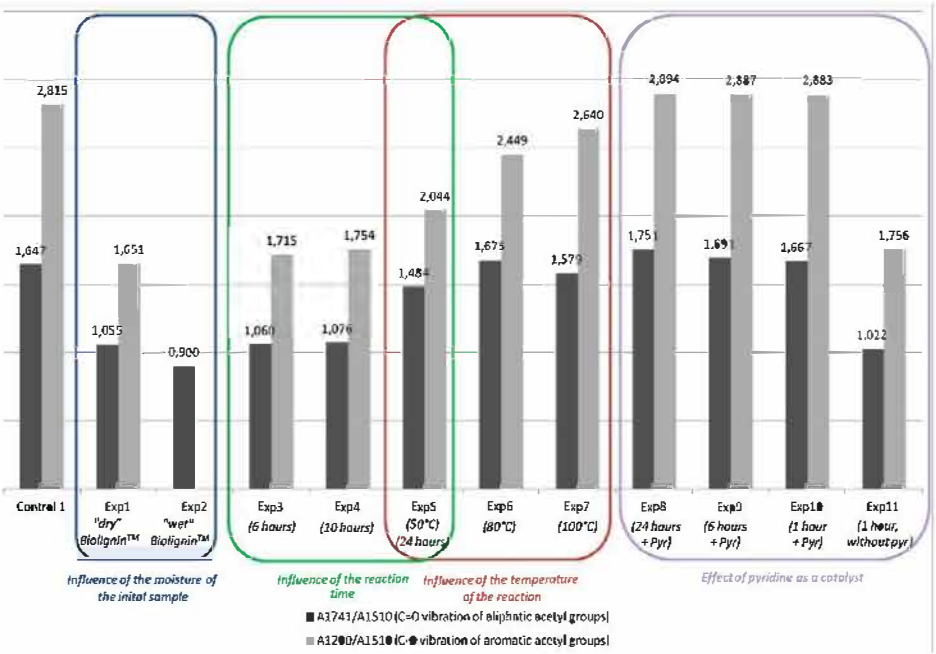

Fig. 3. FTIR absorbance ratios of A_{1741}/A_{1510} and A_{1200}/A_{1510} of acetylated Biolignin™ samples.

and 7, respectively). 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 Exp7 than on the spectra of the previous experiments. A shoulder band at $\nu \approx 1760\,cm^{-1}$ 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 ($\nu \approx 1220\,cm^{-1}$) decreased (not shown) and the broad band corresponding to the hydrogen bonded –OH ($\nu \approx 3400\,cm^{-1}$) had nearly disappeared on the FTIR spectrum of the Biolignin™ 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 Biolignin™ in a sc-CO_2 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 Biolignin™ 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 Biolignin™ sample with a dramatically higher DS of its hydroxyl groups compared to the same experiment without catalyst (Exp8 compared to Exp7, Fig. 3). The DS obtained after 1 and 6 h of reaction (Exp10 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 Biolignin™ 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 (Exp11) was conducted in thesame conditions as Exp10 (1 h, 100 °C, 180 bars) without pyridine. As shown in Fig. 3, the DS of the acetylated Biolignin™ seemed to be lower in absence of pyridine (Exp11 compared to Exp10).

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

The DS observed on acetylated Biolignin™ 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 hindrance 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 Exp8 (with pyridine) was close to the one observed in Exp7 (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 Biolignin™ before acetylation, sc-acetylated Biolignin™ with non-optimized conditions (Exp3, 6 h, 50 °C; 100 bar, without catalyst) and sc-acetylated Biolignin™ with optimized conditions (Exp10, 1 h, 100 °C; 180 bar, with pyridine as a catalyst).

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

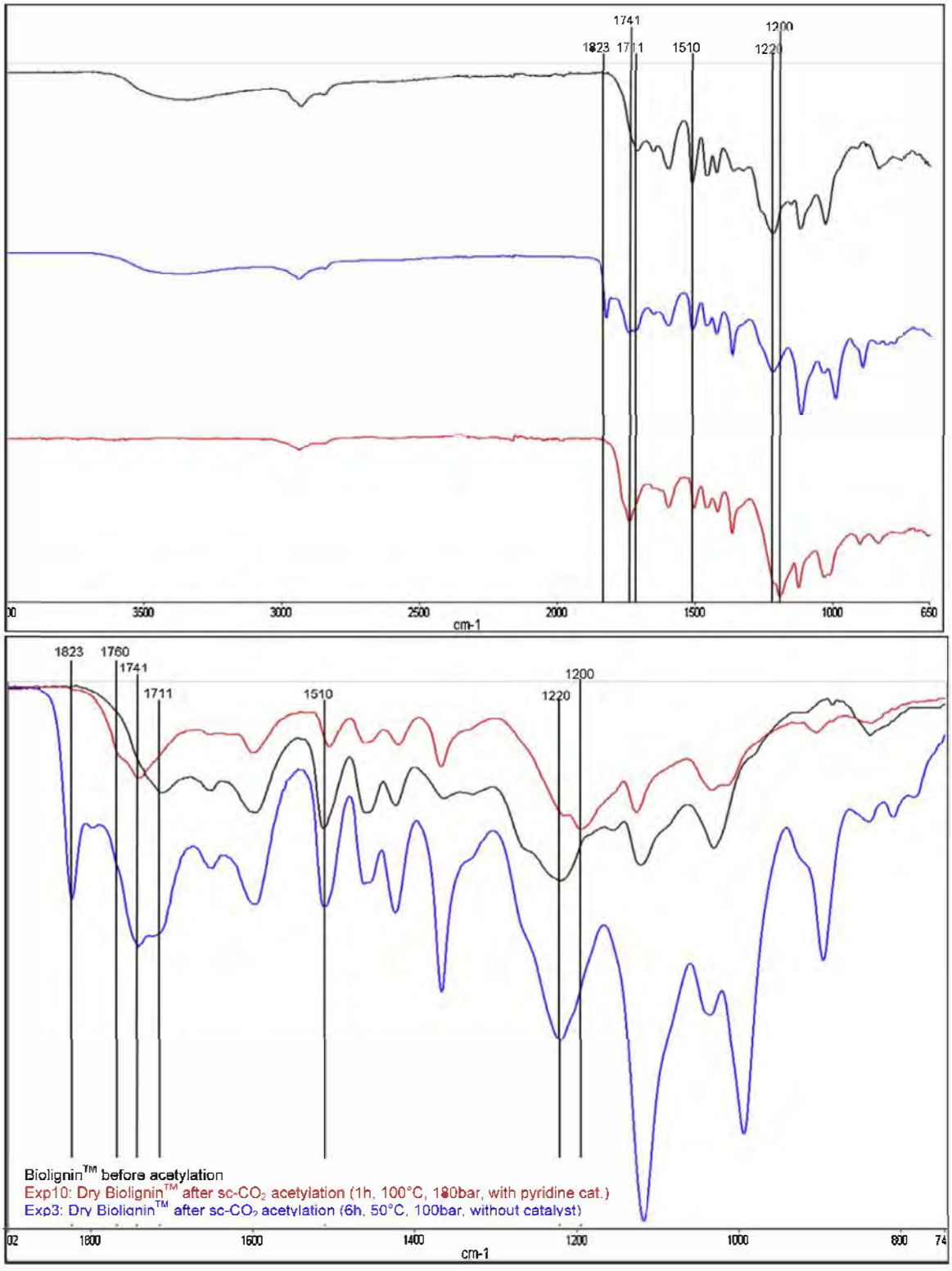

Fig. 4. ATR-FTIR spectra of initial Biolignin™ and acetylated Biolignins™ from Exp3(non-optimized sc-conditions) and 10 (optimized sc-conditions).

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

Table 3
Hydroxyl group contents of unmodified and acetylated Biolignin™ samples as determined by ^{31}P NMR analysis.

		Unmodified Biolignin™	Control 1	Control 2	Control 3	Exp 4	Exp 10	Exp 12
Quantitation (mmol/g of sample)			0.179	0.146	0.216	0.034	0.041	
–COOH	0.238	0.034	0.000	0.000	0.000	0.000	0.000	
Phenolic –OH of *p*-hydroxylphenyl-units (H-units)	0.161	0.000	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	% Residual –OH in H-units		0.238	0.175	0.512	0.062	0.066	
Phenolic –OH of guaiacyl units (G-units)	1.255	0.098	7.8%	19.0%	13.9%	40.8%	4.9%	5.3%
	% Residual –OH in G-units		0.000	0.067	0.209	0.000	0.000	
Phenolic –OH of syringyl units (S-units)	0.772	0.004	0.5%	0.0%	8.7%	27.1%	0.0%	0.0%
	% Residual –OH in S-units		0.272	0.395	0.488	0.173	0.217	
Primary –OH primaires	0.802	0.221	0.000	0.000	0.000	0.000	0.000	
Secondary α-OH of β-*O*-4' linkage (*erythro*)	0.000	0.000	0.034	0.038	0.056	0.012	0.006	
Secondary α-OH of β-*O*-4' linkage (*threo*)	0.151	0.017	0.340	0.545	0.675	1.266	0.248	0.289
Total –OH (mmol/g of sample)		3.141	10.8%	17.4%	21.5%	40.3%	7.9%	9.2%
	% Residual –OH		0.306	0.433	0.545	0.186	0.223	
Total aliphatic OH (mmol/g of sample)	0.953	0.238	70.0%	56.1%	64.1%	43.0%	75.0%	77.2%
	% Residual aliphatic OH	(30.3%)	0.102	0.238	0.242	0.721	0.062	0.066
Total phenolic OH (mmol/g of sampe)		2.188	30.0%	43.7%	35.9%	57.0%	25.0%	22.8%
	% Residual phenolic OH	(69.7%)						

wheat straw lignins (Crestini and Argyropoulos, 1997; Yang et al., 2011) and more specifically for wheat straw Biolignins™ (Delmas et al., 2011; Arshanitsa et al., 2013). The hydroxyl content of Biolignin™ included 0.95 mmol/g of aliphatic hydroxyl groups and 2.19 mmol/g of phenolic hydroxyl groups (Table 3).

The ^{31}P NMR results highlighted the presence of non-acetylated hydroxyls even when the Biolignin™ samples were conventionally acetylated (Table 3). Indeed, after acetylation, Controls 1, 2 and 3 still contained 10.8%, 17.4% and 21.5% of free hydroxyl groups, respectively. More than the half of these free hydroxyls was composed of primary aliphatic hydroxyls. In all conventional esterification procedures and supercritical conditions tested, it seemed nearly impossible to acetylate all aliphatic hydroxyls. The optimized supercritical experiment (Exp10) gave the best results: after reaction, less than 8.0% of the hydroxyl groups were still free (Table 3), i.e., less than when using conventional procedures. However, once again, these free hydroxyls were mainly composed of aliphatic hydroxyls (75.0%). This unexpected result draw attention on the pertinence of the hydroxyl group quantification of Biolignin™ by the very common GC-FID method, often quoted in the literature. Indeed, this technique implies the acetylation (usually in conventional media) of lignin samples before the analysis. 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, ^{31}P 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 seemed 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 actif role as a catalyst but also as a solvent.

According to the ^{31}P 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 sc-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 obtain 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.

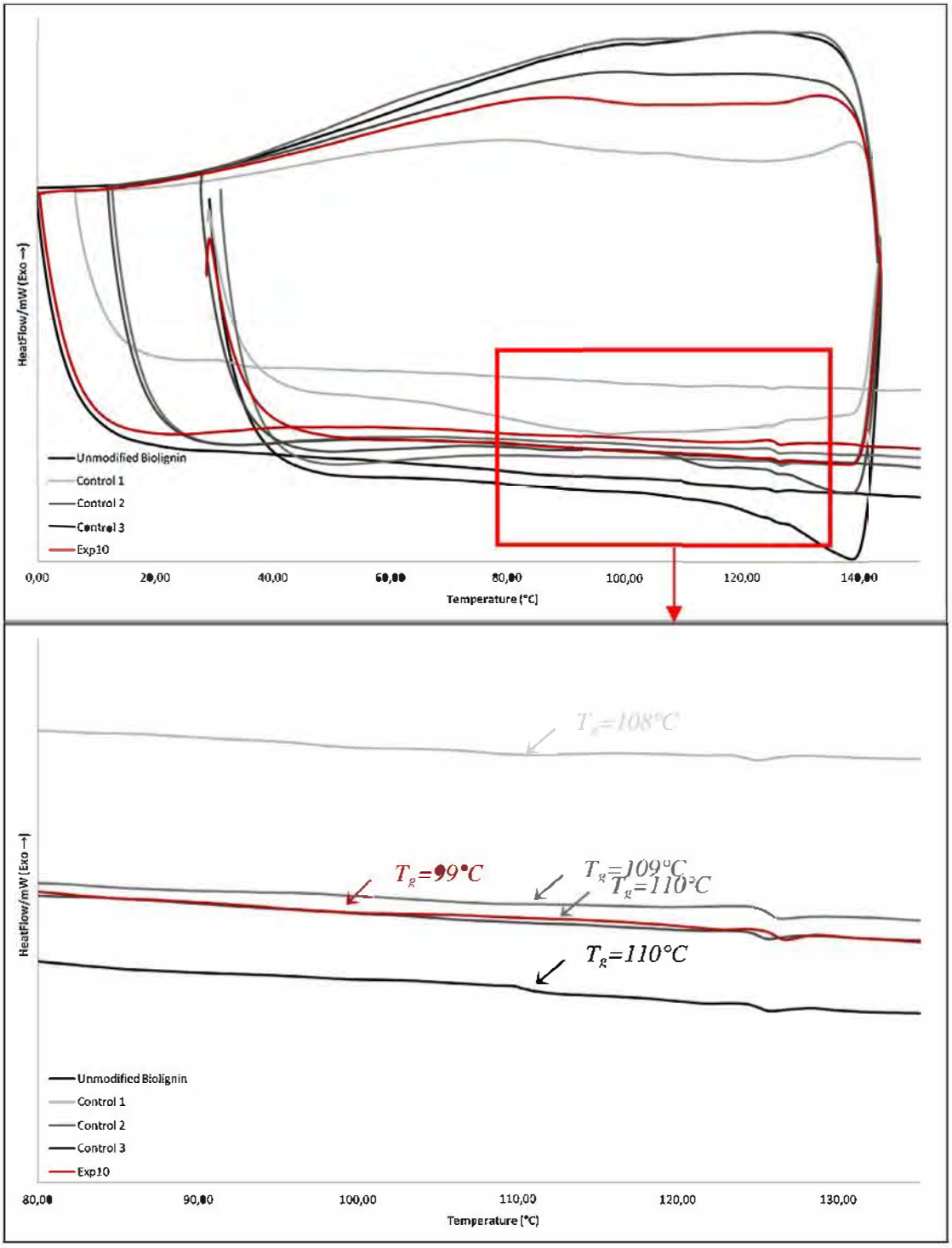

Fig. 5. DSC thermograms of initial and acetylated BioligninTM samples.

These results showed that, in addition to be a quicker and a more user-friendly procedure, the reaction under supercritical conditions allowed a better acetylation of the Biolignin^{TM} 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^{TM} hydroxyl groups compared to Exp10. It appeared that this increase of the temperature did not allow better acetylation. On the contrary, the modified Biolignin^{TM} 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^{TM} was completely different from the one under sc-conditions. Indeed, in conventional procedure, the Biolignin^{TM} sample is insoluble at the beginning. Then, the "modified" Biolignin^{TM} 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^{TM} is recovered as a solid powder.

Under sc-conditions, the Biolignin™/acetylated Biolignin™ 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™ samples had a different behavior: conventionally acetylated Biolignins™ were soluble in a variety organic solvents (acetone, 1,4-dioxane, THF, etc.) while sc-acetylated Biolignins™ were only slightly soluble in these solvents. Some physical properties of the acetylated Biolignin™ 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™ samples*

The thermal properties of the initial and acetylated Biolignin™ 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™ 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™ exhibited a T_g lower than the initial Biolignin™ (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™ 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™ 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 studywould be needed to confirm this hypothesis. It is also possible that the procedure of acetylation directly affect the T_g of the acetylated Biolignin™. 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™ may be lower than the ones of conventionally acetylated Biolignin™. 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 evacuation of carbon dioxide and unreacted anhydride, when return to atmospheric conditions.

On a physical point of view, the acetylation reaction of the Biolignin™ under sc-condition was different than the one obtained by the conventional procedures where the Biolignin™ sample became gradually soluble in the medium until reaching a complete solubility at the end of the reaction. Under sc-condition, the Biolignin™/acetylated Biolignin™ 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™ seemed to be approximately the same. However the procedure induced a different physical behavior. The conventionally acetylated Biolignin™ samples were soluble in a variety of organic solvent (THF, 1,4-dioxane, acetone, etc.) while the acetylated Biolignin™ 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.

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