# Chemical Alterations of Pine Wood Lignin during Heat Sterilization

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This study investigated alterations in the lignin of pinewood (*Pinus sylvestris* L.) during heat sterilization. The Klason lignin content was determined, and the dioxane lignin was isolated. Changes in the dioxane lignin were evaluated by size exclusion chromatography (SEC) as well as Fourier transform infrared (FTIR) spectroscopy. The analyses illustrated an increase in the percentage of extractives and the amount of dioxane lignin yields, while a decrease in the acid-insoluble lignin content was also observed. Changes in the macromolecular characteristics of the dioxane lignin (molecular weight and polydispersity) were not significant ($p < 0.05$); however, they indicated that simultaneous degradation and condensation reactions had occurred. The FTIR spectra of the dioxane lignins showed an increase at 3420 cm$^{-1}$ (O-H stretching vibration), indicating the cleavage of ether bonds. The band at 1715 cm$^{-1}$ (unconjugated C=O stretching) increased after an initial decrease due to the cleavage of the β-alkyl-aryl ether bonds. Also, the band at 1660 cm$^{-1}$ (conjugated C=O groups) increased, which was caused by the cleavage of the α-alkyl-aryl ether bonds.

## INTRODUCTION

Wood is commonly used as an engineering and structural material. Unprotected wood that has been exposed to outdoor conditions undergoes a variety of degradation reactions caused by diverse environmental factors such as light, moisture, heat, oxygen, pollutants, pests, *etc*. (Evans *et al.* 1992; Hon 1994; Teaca *et al.* 2013). There are various ways to protect the wooden parts of buildings, *e.g.* nano-metal impregnation (Huang *et al.* 2015), insecticide treatments (Ahmed *et al.* 2000), fungal metabolites (Yang *et al.* 2007), and thermal modification (Esteves *et al.* 2008).

Thermal modification has been used to improve the physical and mechanical properties of wood for nearly a century (Esteves and Pereira 2009). The interest in thermally modified wood has increased due to its environmental friendliness. Heat-treated wood generally has better dimensional stability, improved biological durability, lower equilibrium moisture content, reduced surface roughness, enhanced weather resistance, a

decorative darker color, and a better decay resistance than natural wood (Alen *et al.* 2002; Shi *et al.* 2007; Icel *et al.* 2015; Kvietková *et al.* 2015).

Thermal modification at lower temperatures is called heat sterilization. It is mainly used to kill pests within solid wood material. The current international standard for the heat sterilization of solid wood requires heating wood to a minimum core temperature of 56 °C for a minimum of 30 min (Wang 2010). Heat sterilization at 120 °C increases the core temperature to 55 °C — 60 °C; after 1 h, the amount of terpenes—attractants for various insects—is decreased (Kacik *et al.* 2012); during the heat sterilization of pinewood, a slight loss in the mass, an increase in the percentage of extractives, and a decrease in the amount of lignin and polysaccharides were observed. Hemicelluloses are degraded approximately twice as fast as cellulose. The degree of the polymerization of cellulose decreases by approximately 10%, and it increases in the holocellulose by about 8% as a result of the faster degradation of shorter hemicellulose chains (Kacik *et al.* 2015).

One of the main components of wood is lignin. Lignin has complex, condensed, amorphous structures that allow it to resist external physical, chemical, and/or biological forces (Kim *et al.* 2014). Lignin polymers consist of three types of phenylpropanoid units of various proportions: hydroxyphenyl (H), guaiacyl (G), and syringyl (S). Lignin protects cellulose by enclosing it in a physical matrix (Zeng *et al.* 2013).

A disadvantage of thermal modification is that it diminishes the mechanical properties of wood including modulus of rupture (MOR) and modulus of elasticity (MOE). This weakness limits the possible applications of wood treated by this method. The reduction of these properties is correlated to the severity of the time and temperature of the treatment (Candelier *et al.* 2015). An increase in lignin content reduces the tensile modulus and tensile strength of the composites; however, the flexural and torsional modulus are improved with increasing lignin content (Faruk and Sain 2015).

Most research has focused on the mechanical, physical, and chemical properties of wood treated at higher temperatures rather than at lower temperatures. The alterations in saccharides during the heat sterilization of pinewood have been reported previously (Kacik *et al.* 2015), and this study investigates the changes occurring in pinewood lignin treated under the same conditions.

## EXPERIMENTAL

### Materials

For this study, 100-year-old Scots pine sapwood (*Pinus sylvestris* L.) was used. Wood for samples was taken from Karlstejn Castle, Czech Republic. Three boards measuring 1000 mm × 180 mm × 25 mm (length × width × thickness) were conditioned at 20 °C ± 2 °C and 65% ± 5% relative humidity to a moisture content of about 12%. Each of the boards was divided into three identical parts. One part was saved as a reference, and the other two were thermally treated.

### Methods

*Heat treatment*

Heat treatment was applied to the experimental samples in a laboratory type heating oven Memmert UNB 200 (Fisher Scientific, Loughborough, UK), which was controlled to an accuracy of ± 1 °C under atmospheric pressure at the temperature of 60 °C and 120 °C. Once the target temperature was reached, the temperature was held constant for 10 h

(Kacik *et al.* 2015). The temperature in the core reached the required temperatures within 1 h (Hrcka and Babiak 2012).

*Chemical analyses*

The reference sample, which was left untreated and denoted as 20 °C, and the thermally modified pinewood samples were mechanically disintegrated to sawdust using a lab mill POLYMIX PX-MFC 90 D (KINEMATICA AG, Luzern, Switzerland). Particles of 0.5 to 1.0 mm were extracted from the Soxhlet apparatus (Sigma-Aldrich, Munich, Germany) using a mixture of ethanol and toluene according to ASTM D1107-96 (2013). The lignin content was determined according to ASTM D1106-96 (2013). Dioxane lignin was isolated from 10 g of each sample in 200 mL of a dioxane-water (9:1) mixture for 5 h at 80 °C (Kosikova *et al.* 2006).

*Size exclusion chromatography (SEC)*

The molecular weight distribution (MWD) of lignin was measured by a modified SEC method (Šurina *et al.* 2015). The isolated dioxane lignin was dissolved in dimethyformamide (DMF) (c = 5 mg/mL) and filtered through a Puradisc 25 NYL filter (Whatman International, Maidstone, UK) with a pore size of 0.45 μm. The separation was performed at 35 °C with LiBr (0.005 M) in DMF at a flow rate of 1 mL/min on a POLAR-M column (7.5 mm × 300 mm) (Agilent, Santa Clara, CA, USA). A differential refractometer (RI) and diode array detector (DAD) at 280 nm were used as the detectors. Data were acquired with Chemstation software (Agilent) and analyzed with the Clarity GPC module (DataApex, Prague, Czech Republic). The system was calibrated with polystyrene standards from 500 to 98,900 g/mol (Tosoh, Tokyo, Japan). All SEC results represent the mean of two different samples. Each sample was run in two replicates in two experiments, for a total of four runs for each sample.

*Fourier transform infrared (FTIR) spectroscopy*

FTIR spectra were recorded on a Nicolet iS10 spectrometer (Thermo Fisher Scientific, Walham, MA, USA) equipped with Smart iTR using attenuated total reflectance (ATR) sampling with a ZnSe crystal accessory (Thermo Fisher Scientific, Walham, MA, USA). The spectra were acquired by accumulating 64 scans at a resolution of 4 $cm^{-1}$ in an absorbance mode (A) from 4000 $cm^{-1}$ to 400 $cm^{-1}$ and normalized to the maximum at around 1600 $cm^{-1}$.

*Statistical analysis*

For all parameters, multiple comparisons were first subjected to an analysis of variance (ANOVA), and the significant differences between the average values of the control and treated samples were determined using Duncan's multiple range test with a *p*-value of 0.05.

## RESULTS AND DISCUSSION

During thermal treatment, the amount of lignin decreased significantly (Table 1; Fig. 1). Previously it was shown that lignin content increases after a severe thermal treatment (Nuopponen *et al.* 2004; Windeisen and Wegener 2008; Ates *et al.* 2009). Lignin is generally more heat-resistant than cellulose and hemicelluloses; however, at lower

temperatures, part of lignin and polysaccharides amounts can be converted to the extractives (Table 1). During the thermal degradation of hemicelluloses, lignin-carbohydrate connections are also cleaved (Pfriem and Wagenfuehr 2008), which depolymerizes the non-carbohydrate-bonded lignin fraction, yielding simple phenolic compounds.
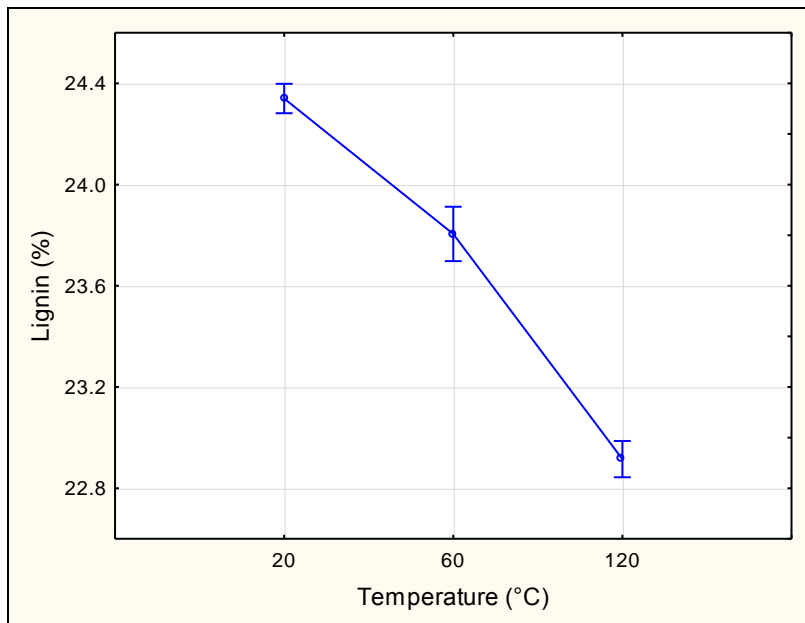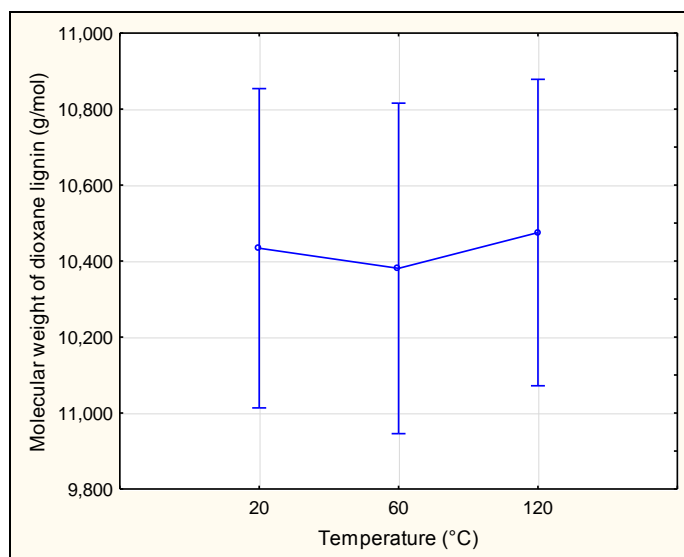


**Fig. 1.** Influence of temperature on lignin content with 95% confidence intervals
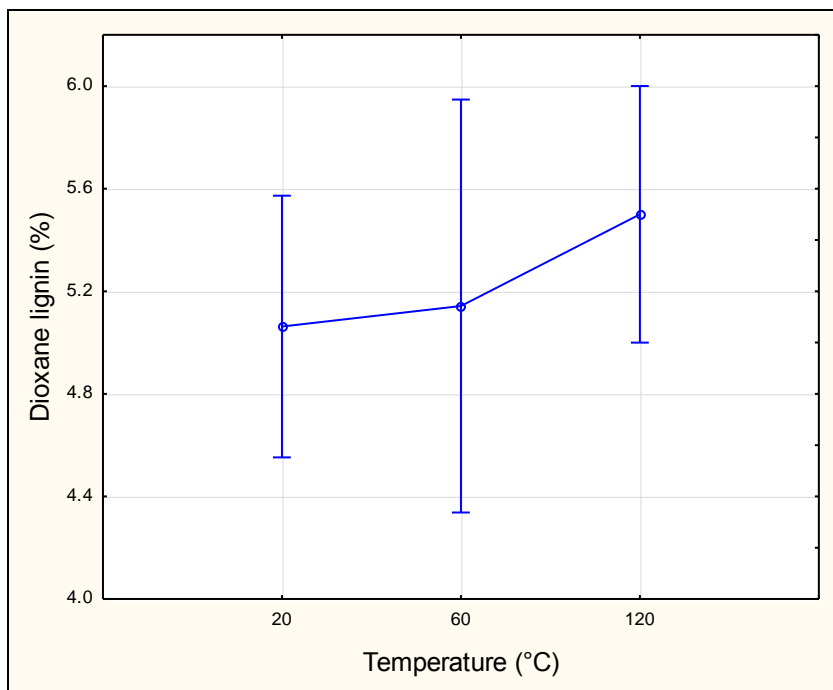


**Fig. 2.** Influence of temperature on the yield of dioxane lignin with 95% confidence intervals

To study the depolymerization and condensation reactions of lignin during heat sterilization, the average molecular weights ($M_w$, $M_n$, $M_z$, and $M_p$) and polydispersity (PD = $M_w/M_n$) were determined by SEC (Table 1). The changes in the yields and macromolecular characteristics of the pine dioxane lignin during heat sterilization were insignificant (Table 1; Figs. 2 and 3); however, some alterations in lignin were observed. A negligible decrease in $M_w$ at 60 °C was followed by an increased $M_w$ at 120 °C, a decrease in $M_p$, and an increase in polydispersity indicated the occurrence of simultaneous degradation and condensation reactions. The peak maximum shifted to a lower molecular weight range, indicating the decomposition of the lignin at both temperatures (Fig 4). In contrast, the MWD curve of the lignin heated at 120 °C also showed a slight increase in $M_z$, corresponding to a higher molecular weight as a result of lignin condensation. These findings confirmed that depolymerization, side chain cleavage, recondensation, and carbonization occur during exposure to heat (Li *et al.* 2007; Kacikova *et al.* 2008; Shen *et al.* 2010; Kim *et al.* 2014).



**Fig. 3.** Influence of temperature on the molecular weight of dioxane lignin with 95% confidence intervals

**Table 1.** Extractives, Lignin, and Dioxane Lignin Yields, and Macromolecular Characteristics of Dioxane Lignin*

| T (°C) | Extractives (%) | Lignin (%) | Dioxane Lignin (%) | $M_w$ (g/mol) | $M_n$ (g/mol) | $M_z$ (g/mol) | $M_p$ (g/mol) | PD |
|---|---|---|---|---|---|---|---|---|
| 20 | 5.80 ± 0.08 | 24.34 ± 0.04 | 5.06 ± 0.32 | 10,434 ± 264 | 2,849 ± 142 | 47,977 ± 1,331 | 3,317 ± 63 | 3.67 ± 0.09 |
| 60 | 7.94 ± 0.09 | 23.81 ± 0.07 | 5.14 ± 0.51 | 10,381 ± 273 | 2,787 ± 132 | 46,904 ± 1,005 | 3,269 ± 83 | 3.73 ± 0.09 |
| 120 | 12.47 ± 0.10 | 22.97 ± 0.05 | 5.50 ± 0.31 | 10,475 ± 253 | 2,748 ± 105 | 49,993 ± 1,937 | 3,128 ± 59 | 3.81 ± 0.05 |

*Note: The data shown are means ± standard deviations. PD = $M_w/M_n$
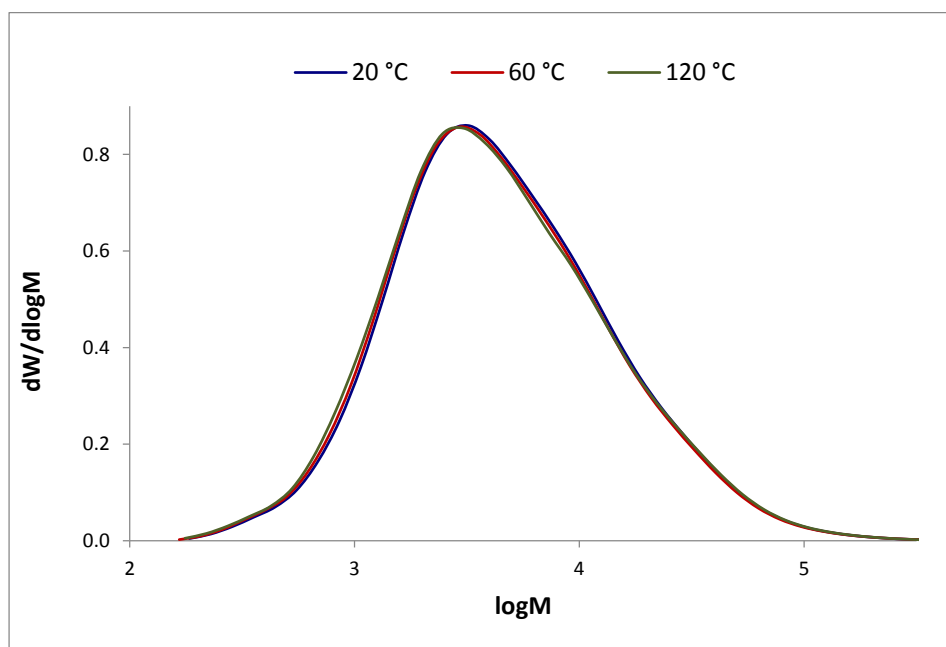
**Fig. 4.** Molecular weight distribution of pinewood lignin before and after heat sterilization

Duncan's multiple range test p-values (Tables 2, 3, 4) presents the same results, as can be seen from Figs. 1, 2, and 3. The yield of Klason (acid insoluble) lignin changes significantly, alterations of dioxane lignin yield and its molecular weight are not significant.

**Table 2.** Duncan's Multiple Range Test p-values for Klason Lignin Yield

| Averages (%) | 24.34 | 23.81 | 22.92 |
|---|---|---|---|
| Temperature (°C) | 20 | 60 | 120 |
| 20 | | 0.000 | 0.000 |
| 60 | 0.000 | | 0.000 |
| 120 | 0.000 | 0.000 | |

**Table 3.** Duncan's Multiple Range Test p-values for Dioxane Lignin Yield

| Averages (%) | 5.06 | 5.14 | 5.50 |
|---|---|---|---|
| Temperature (°C) | 20 | 60 | 120 |
| 20 | | 0.779 | 0.164 |
| 60 | 0.779 | | 0.228 |
| 120 | 0.164 | 0.228 | |

**Table 4.** Duncan's Multiple Range Test p-values for Dioxane Lignin Molecular Weight

| Averages (g/mol) | 10,434 | 10,381 | 10,475 |
|---|---|---|---|
| Temperature (°C) | 20 | 60 | 120 |
| 20 | | 0.783 | 0.830 |
| 60 | 0.783 | | 0.640 |
| 120 | 0.830 | 0.640 | |

The infrared spectra of the dioxane lignin showed an increase in the intensity of the band at 3420 cm$^{-1}$, corresponding to the O-H stretching vibration from the alcohols (3425 cm$^{-1}$ to 3400 cm$^{-1}$), indicating the cleavage of ether bonds (Fig. 5; Table 5), as previously observed (Esteves *et al.* 2013).

The band at 1715 cm$^{-1}$, which illustrates the carbonyl stretching of unconjugated β-ketone and conjugated acid/esters, increased after an initial decrease, due to the cleavage of the β-alkyl-aryl ether bonds and the production of C=O bonds within the lignin (Kosikova *et al.* 2006; Esteves *et al.* 2013). The band at 1660 cm$^{-1}$, attributed to the stretching of conjugated aryl carbonyl (1675 cm$^{-1}$ to 1660 cm$^{-1}$), also increased, likely due to the cleavage of the α-alkyl-aryl ether bonds. However, at higher temperatures, this peak decreased as a result of reactions between α-sites of the side chains in the lignin and in the neighboring benzene nuclei, forming diphenylmethane structures (Funaoka *et al.* 1990; Gierer 2009). An initial decrease was followed by an increase in the 1715 cm$^{-1}$ band relative to the 1660 cm$^{-1}$. Thus, the non-conjugated C=O groups increased relative to the conjugated groups. Gonzalez *et al.* (2009) and Esteves *et al.* (2013) described similar results.

The bands at 1510 cm$^{-1}$ and 1220 cm$^{-1}$ were assigned to a guaiacyl unit (1515 cm$^{-1}$ to 1510 cm$^{-1}$ vibration of aromatic skeleton, 1220 cm$^{-1}$ to 1230 cm$^{-1}$ guaiacyl unit breathing with C=O stretching vibration); the intensities of these bands decreased. The bands at 1460 cm$^{-1}$ and 1370 cm$^{-1}$ decreased as well. The band at 1470 cm$^{-1}$ to 1460 cm$^{-1}$ showed asymmetric C-H deformation, and the 1370 cm$^{-1}$ to 1365 cm$^{-1}$ band illustrated a symmetric C-H deformation. The vibration of the aromatic skeleton at 1420 cm$^{-1}$ decreased, as did the C-O deformation in the primary alcohols at 1030 cm$^{-1}$. These changes were caused by lignin degradation and the cleavage of methoxyl groups during heat sterilization. Lignin demethoxylation during the heat treatment of pine, spruce, and eucalyptus wood has been observed. However, this reaction occurs more in hardwoods than in softwoods because syringyl units are generally less condensed by C-C bonds than guaiacyl monomers and are more easily liberated by thermal degradation (Faix *et al.* 1990; Kosikova *et al.* 2006; Esteves *et al.* 2013).

**Table 5.** FTIR Absorbance of Lignin Samples Isolated from Pinewood before and after Heat Sterilization*

| Temperature (°C) | Wavenumber (cm$^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3420 | 1715 | 1660 | 1510 | 1460 | 1450 | 1420 | 1370 | 1030 |
| 20 | 0.573 | 0.279 | 0.404 | 2.327 | 1.411 | 1.386 | 1.092 | 0.424 | 2.789 |
| 60 | 0.596 | 0.267 | 0.414 | 2.317 | 1.405 | 1.376 | 1.083 | 0.426 | 2.777 |
| 120 | 0.606 | 0.299 | 0.424 | 2.247 | 1.365 | 1.354 | 1.069 | 0.409 | 2.685 |

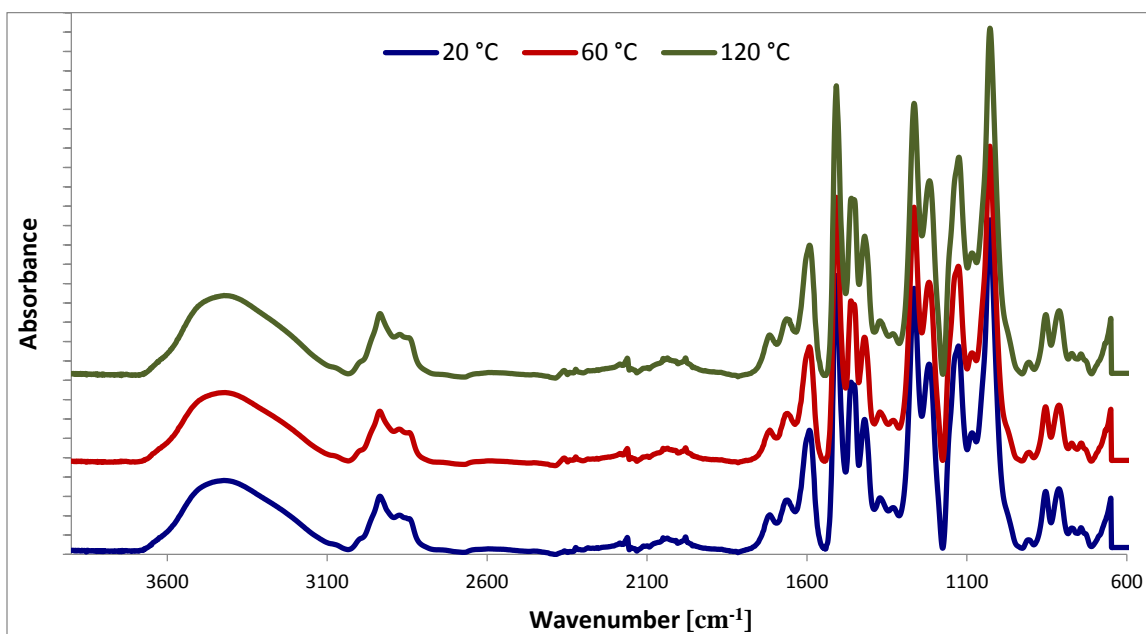*Relative to the absorbance of peaks at 1590 cm$^{-1}$

**Fig. 5.** FTIR spectra of lignin samples isolated from pinewood before and after heat sterilization

## CONCLUSIONS

1. During heat sterilization, a significant increase ($p < 0.05$) in the percentage of extractives (from 5.80% to 12.47%) and dioxane lignin yields (from 5.06% to 5.50%) was observed. The acid-insoluble lignin content decreased from 24.34% to 22.97%.

2. Simultaneous degradation and condensation reactions at higher temperatures took place; however, changes in the macromolecular characteristics of the dioxane lignin, *i.e*., the molecular weight and polydispersity, were not significant ($p < 0.05$).

3. FTIR spectra of dioxane lignin showed an increase in the number of hydroxyl and carbonyl groups due to the cleavage of the alkyl-aryl ether bonds and the demethoxylation occurring at higher temperatures.

## ACKNOWLEDGMENTS

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