

CHARACTERIZATION OF MACROMOLECULAR TRAITS OF LIGNIN FROM HEAT TREATED SPRUCE WOOD BY SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

Lignin composition, functionality, purity, molecular weight distribution and degree of cross-linking are of great interest because of their impact on physicochemical properties and influence on its potential applications. In this study, dioxane lignin from spruce wood thermally treated at various temperatures (100, 150, 200, 220, 240, 260, 280 and 300 °C) and duration (1, 3 and 5 hours) was investigated by size exclusion chromatography (SEC) using 0.005 M LiBr in DMF on two POLAR-M columns. With increasing temperature and duration of treatment the degradation and condensation reactions take place in lignin. At the temperatures till 200–240 °C, the degradation and condensation reactions take place simultaneously, but the lignin macromolecule condensation is predominant, and the molecular weights increase. At the temperatures over 240 °C, the decomposition of high molecular fractions of lignin takes place. Under mild conditions of thermal treatment, the extraction of lignin from wood increased, and with increased severity of treatment it decreased rapidly.

Key words: spruce wood, dioxane lignin, size exclusion chromatography, molecular weights, polydispersity.

INTRODUCTION

As lignin constitutes 15–40% of dry weight of woody plants, it is the most abundant aromatic polymer on the earth and the second most abundant organic polymer after cellulose. Lignin consists of three phenylpropanoid monomers which are sinapyl alcohol, coniferyl alcohol and *p*-coumaryl alcohol. Each of these monomers gives rise to different aromatic nuclei in the lignin structures and their structural complexity due to the simultaneous presence of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl units (S). The amount of these compounds allows establishing the relationship between units S, G and H, and the lignin origin (hardwood, softwood and annual plants) (DOS SANTOS *et al.* 2014, JABLONSKY *et al.* 2016). Lignin's composition, functionality, purity, molecular weight distribution and degree of cross-linking largely determine its physicochemical properties and influence its potential applications (KACIK *et al.* 2014). The development of reliable standard methods for determination of the molar mass distribution is not only relevant for designing technical lignins for specific applications, but also for monitoring and elucidating delignification and pulping processes (BAUMBERGER *et al.* 2007). Many methods to determine the molecular mass of lignin have been developed, e.g. size exclusion chromatography (SEC), light

scattering (LS), vapor pressure osmometry (VPO), ultrafiltration, mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy (ZHUANG 2010, GELLERSTEDT 1992, PLA 1992ab; EVTUGUIN *et al.* 1999, NORGREN, LINDSTRÖM 2000; ZENG *et al.* 2013). The molecular weight of lignin depends on the method of extraction and its ranges from 265 to 50,000,000 (AL-MAMUN *et al.* 2015).

Among these methods for molecular mass determination, SEC has several advantages: it is readily available and provides information for a wide range of molecular masses in a single analysis step. The main parameters obtained by SEC are: weight-average molecular weight (M_w), number-average molecular weight (M_n), molecular weight at peak maximum (M_p), molecular weight z average (M_z). From these parameters useful characteristics of polymers can be calculated, e.g. polydispersity ($PD = M_w/M_n$ or M_z/M_w), degree of polymerization ($DP_w = M_w/M_0$ or $DP_n = M_n/M_0$, where M_0 is the molecular weight of the monomer unit). The constancy of the ratio of the average degree of polymerization (DP_z/DP_w) and/or (DP_{z+1}/DP_w) are an indication of the homogeneity of the depolymerization and their changes indicates the course of different reaction types, e.g. oxidation and cross-linking (ZOU *et al.* 1994, KACIKOVA, KACIK 2009, KACIK *et al.* 2009).

Thermal modification has been used to improve the physical and mechanical properties of wood for nearly a century (ESTEVEZ, PEREIRA 2009). The interest in thermally modified wood has recently increased due to the decreased production of durable raw wood material, the increased interest in durable construction materials, and legislative changes that restrict the use of toxic substances (CEKOVSKA *et al.* 2017, OSVALD, GAFF 2017). However, more severe conditions during thermal modification cause the degradation of hemicelluloses and lignin, resulting in the production of water, carbon dioxide, formic acid, acetic acid, and other substances that can be involved in condensation reactions, and chromophore groups are formed. Thermal processes also affect some physical and mechanical properties (dimensional stability, hygroscopicity, durability, MOR—modulus of rupture, MOE—modulus of elasticity, etc.) (KACIKOVA *et al.* 2013, PFRIEM, WAGENFUEHR 2008, GONZALES-PENA *et al.* 2009, ICEL *et al.* 2015).

The aim of this work was to determine the effect of the temperature and duration of thermal loading on macromolecular traits of spruce lignin because of its influence on mechanical properties of thermally modified wood.

MATERIALS AND METHODS

Materials

Two hundred and forty samples of Norway spruce (*Picea abies* L.) wood with dimensions 150 mm × 10 mm × 10 mm (length × width × thickness) were prepared. Thirty samples were used as a reference, the rest was thermally treated.

Methods

Heat treatment

Heat treatment was conducted using 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 temperatures of 100 °C, 150 °C, 200 °C, 220 °C, 240 °C, 260 °C, 280 °C, and 300 °C for 1, 3, and 5 hours. The untreated sample was denoted as 20 °C. All samples were mechanically disintegrated to sawdust using a lab mill POLYMIX PX-MFC 90 D (KINEMATICA AG, Luzern, Switzerland).

Dioxane lignin preparation

Particles below 0.355 mm were extracted in the Soxhlet apparatus (Sigma Aldrich, Munich,

Germany) with a mixture of ethanol and toluene according to the ASTM D1107-96 (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 (KACIKOVA *et al.* 2008).

Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) of dioxane lignins was performed by a modified method (SURINA *et al.* 2015). Dioxane lignin samples were dissolved in dimethylformamide (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 two POLAR-M columns (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 was 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 duplicates.

Statistical analysis

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

RESULTS AND DISCUSSION

Dioxane lignin yields

The amount of lignin that can be extracted with dioxane depends to a large extent on the degree of its condensation (SOLAR, KACIK 1995, KACIK *et al.* 2007). The results of dioxane lignin yields (Tab. 1) show that, under mild conditions of thermal treatment, the yield of lignin from wood increased, and with increased severity of treatment rapidly decreased. The slight increase of dioxane lignin yields was observed at heat sterilization of pine wood in the temperatures up to 120 °C (KACIK *et al.* 2016). However, both degradation and condensation reactions occur in the lignin at higher temperature, resulting in changes in molecular weights as evidenced by the results of size exclusion chromatography (SEC) (Fig. 1–6).

Tab. 1 Dioxane lignin yields from untreated and thermally treated wood (% odw).

Temperature (°C)	Time of thermal treatment (h)		
	1	3	5
20	4.44		
100	4.75	3.67	3.53
150	4.95	4.13	4.27
200	6.17	8.00	9.11
220	8.09	9.38	8.34
240	7.32	6.83	8.01
260	4.49	4.06	3.47
280	3.06	1.84	1.60
300	0.71	-*	-*

*Under the given conditions, the samples started to burn and the lignin was not isolated.

Molecular weights changes Changes in molecular weight and molecular weight distribution of lignin (Fig. 1–3) can provide important insights into fragmentation and cross-

linking reactions during thermal treatment. The weight-average molecular weight (M_w) of the untreated lignin was 6,581 g/mol. After thermal treatment, M_w increased as higher temperature was applied, with the highest value at the temperature of 200 °C ($M_w = 9,569$ g/mol), then decreased to $M_w = 3,132$ g/mol at 260 °C and 5 hours of modification. Similarly, ZHANG *et al.* (2017) found out that cross-linking reactions in poplar lignin were predominant during the hot-pressing process as the molecular weight of lignin increased at elevated temperature up to 180 °C. Generally, the cleavage of linkages, such as β -O-4' linkages, can result in a decrease of the molecular weight of lignin; on the other hand, condensation reactions usually lead to an increase in the molecular size (FARAVELLI *et al.* 2010, ZHANG *et al.* 2017).

Depolymerization and condensation reactions of lignin during heat treatment, the average molecular weights (M_w , M_n , M_z , and M_p) and polydispersity index ($PD = M_w/M_n$) were investigated by SEC. The changes in the yields and macromolecular characteristics of the spruce dioxane lignin during heat treatment were significant. From molecular weight distribution (MWD) curves and from statistical results is evident the shift of weight-average molecular weight (M_w), molecular weight at peak maximum (M_p), and molecular weight z average (M_z) to higher values at the temperatures of 200–240 °C for all duration of treatment. These results indicate cleavage of bonds in lignin and repolymerization reactions resulting in an increase in molecular size and a more heterogeneous lignin structure. Similar effect was observed at steam explosion of aspen wood in the interval of 185–220 °C (LI *et al.* 2007). These findings confirmed that depolymerization, side chain cleavage, recondensation, and carbonization occur during exposure to heat (KACIKOVA *et al.* 2008, KIM *et al.* 2014, SHEN *et al.* 2010).

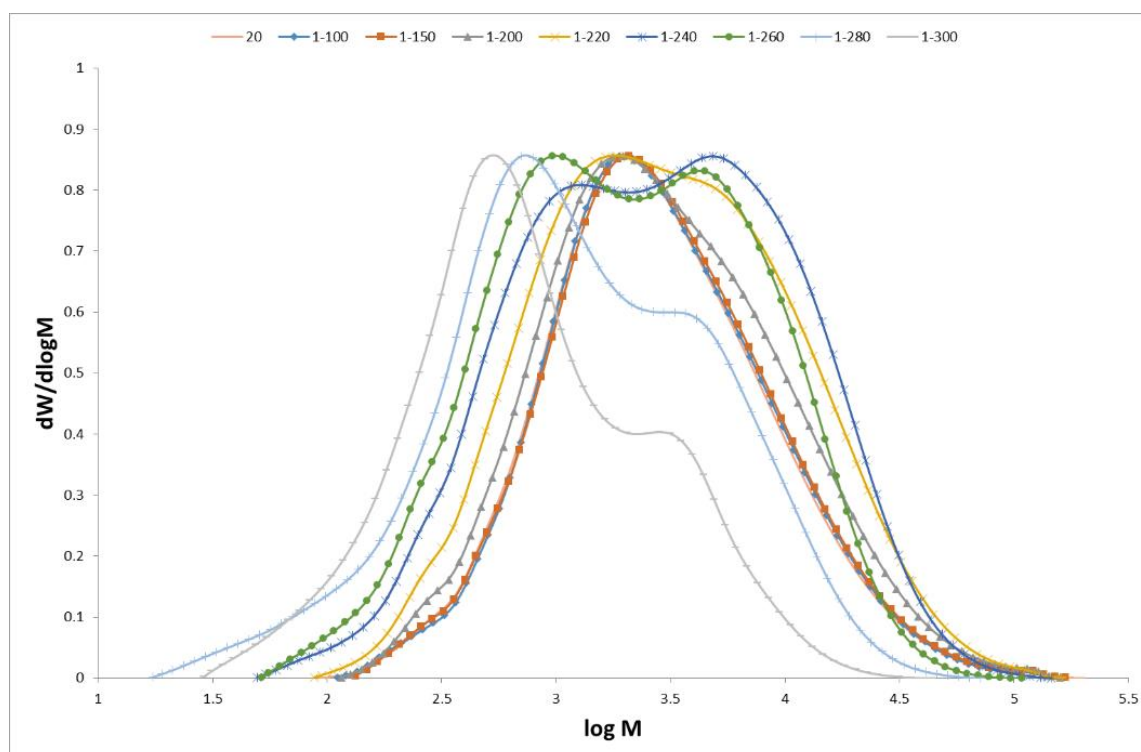


Fig. 1 Influence of 1-hour thermal treatment on molecular weights lignin distribution.

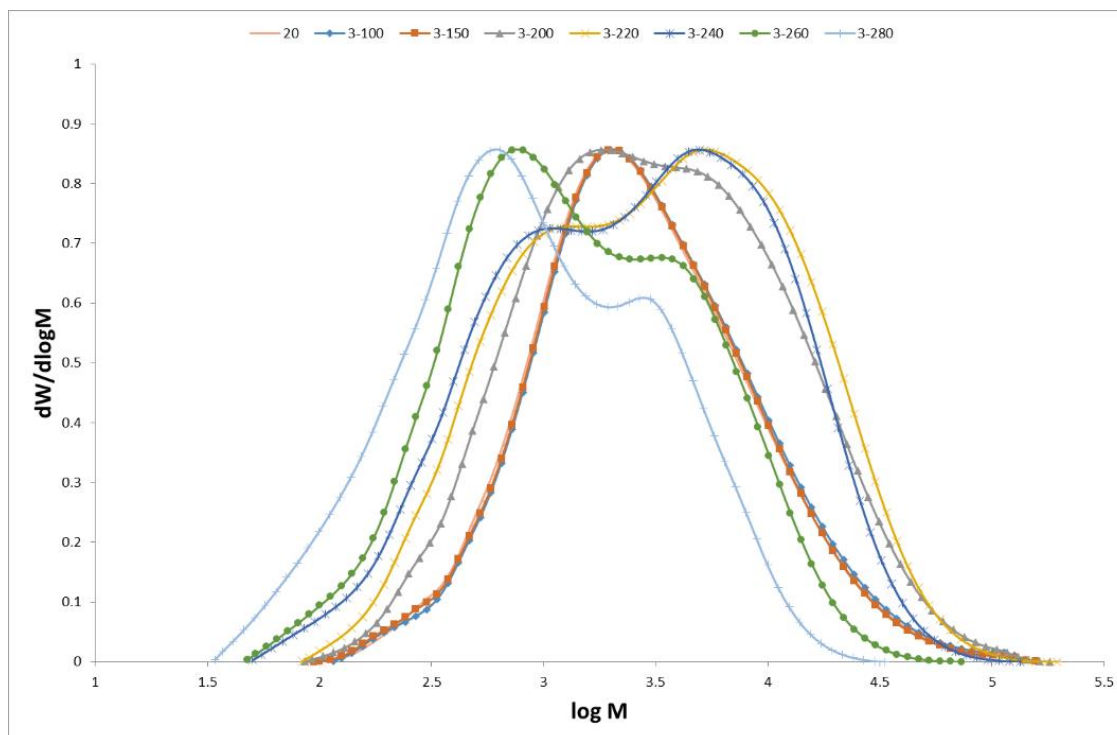


Fig. 2 Influence of 3-hour thermal treatment on molecular weights lignin distribution.

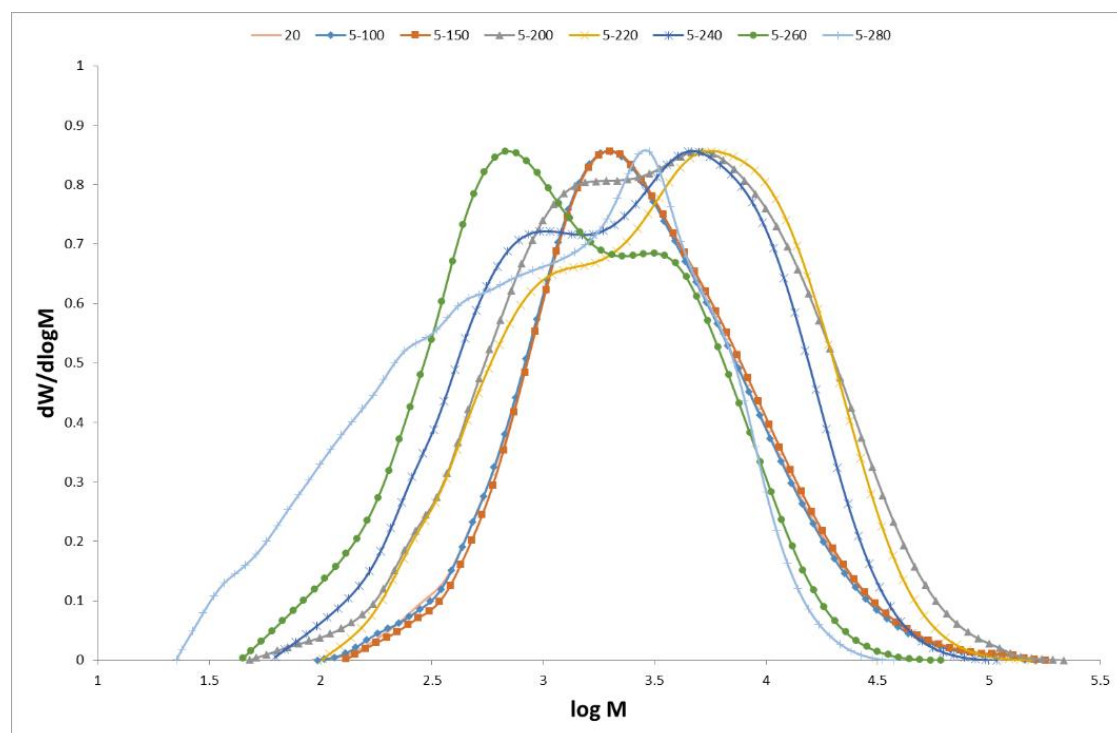


Fig. 3 Influence of 5-hour thermal treatment on molecular weights lignin distribution.

Statistical analysis

Significant ($p < 0.05$) changes in chemical composition of wood samples were observed at all treatment conditions (Fig. 4–7). Significant influence on changes of dioxane lignin macromolecular characteristics have both temperature and treatment duration, and their mutual effect.

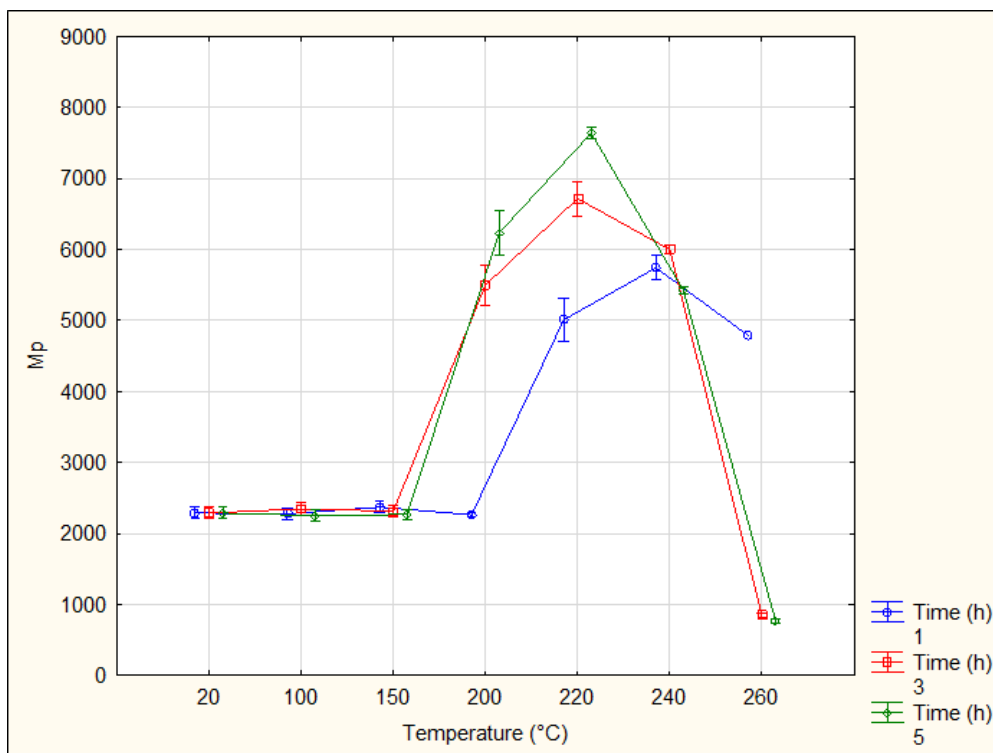


Fig. 4 Influence of temperature on lignin molecular weight at peak maximum (M_p) with 95% confidence intervals.

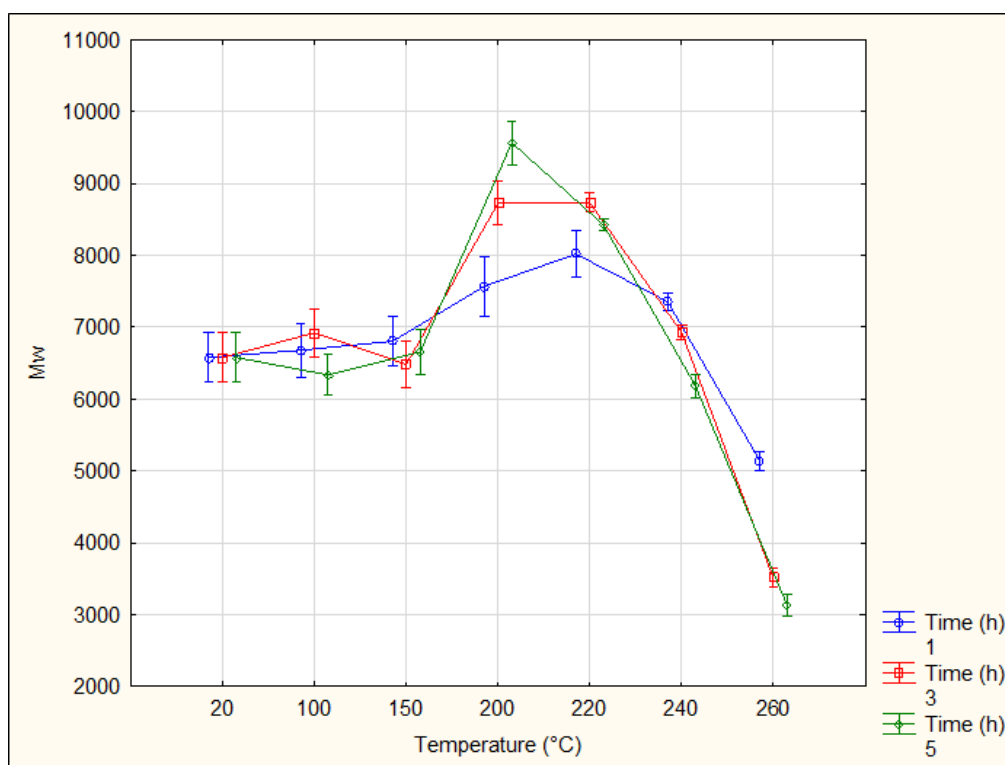


Fig. 5 Influence of temperature on lignin weight-average molecular weight (M_w) with 95% confidence intervals.

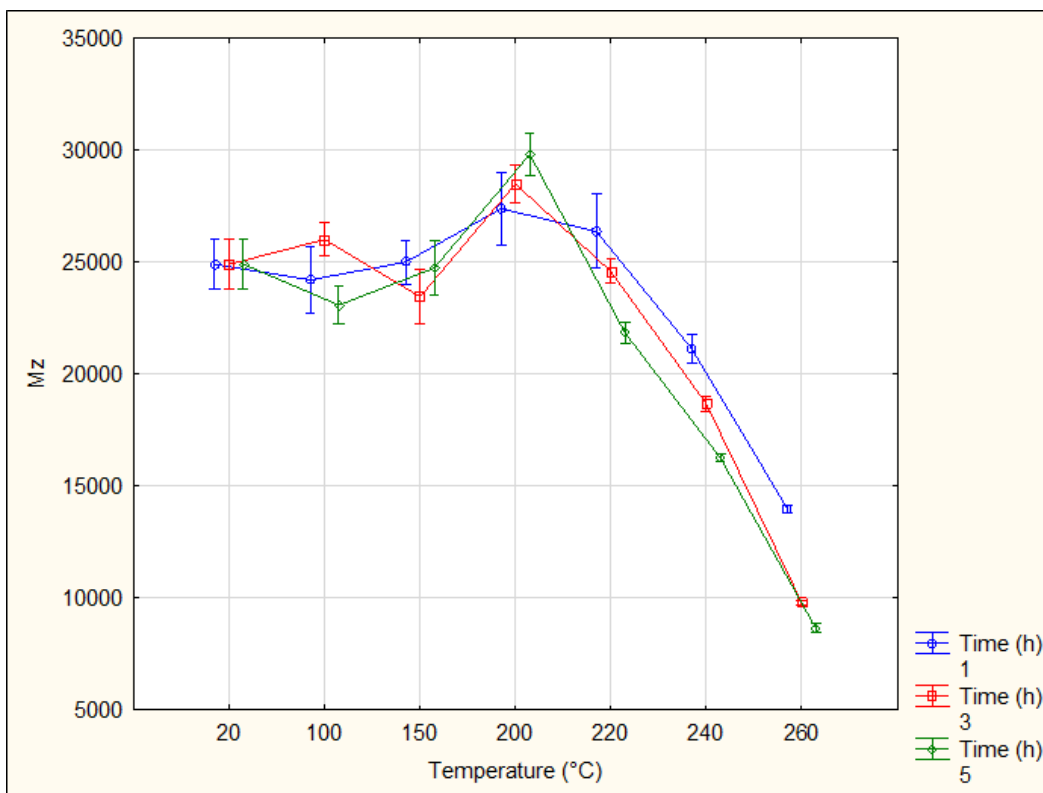


Fig. 6 Influence of temperature on lignin molecular weight z average (M_z) with 95% confidence intervals.

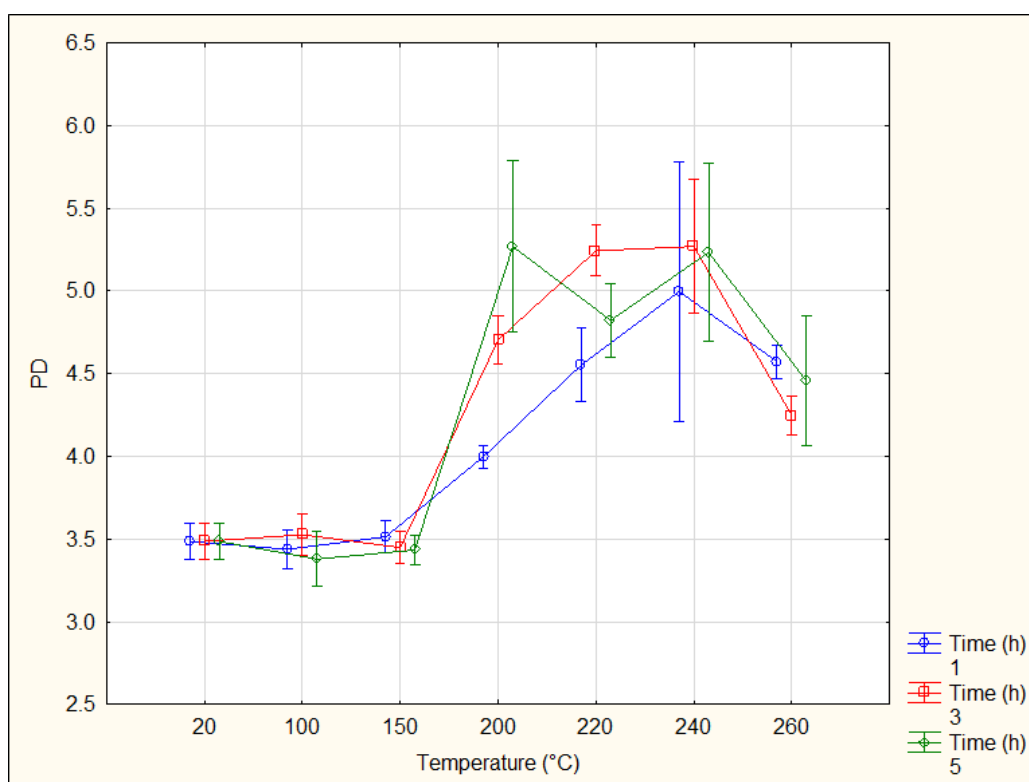


Fig. 7 Influence of temperature on lignin polydispersity index (PD) with 95% confidence intervals.

CONCLUSIONS

In this study, dioxane lignin from spruce wood thermally treated at various temperatures (100, 150, 200, 220, 240, 260, 280, and 300 °C) and duration (1, 3, and 5 hours) was analyzed by size exclusion chromatography.

Lignin is thermally stable to the temperature of 150 °C. With increasing temperature and duration of treatment, the degradation and condensation reactions take place in lignin. Condensation reactions predominate at the temperatures up to 240 °C, and the molecular weights increase in lignin extracted via dioxane. At the temperatures over 240 °C, the decomposition of high molecular fractions of isolated lignin takes place. Under mild conditions of thermal treatment, the extraction of lignin from wood increased, and with increased severity of treatment, it rapidly decreased.

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