

SEC analysis of the molar mass of lignin isolated from poplar (*Populus deltoides x maximowiczii*) and Scots pine (*Pinus sylvestris* L.) wood

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Abstract: SEC analysis of the molar mass of lignin isolated from poplar (*Populus deltoides x maximowiczii*) and Scots pine (*Pinus sylvestris* L.) wood. The aim of the study was to analyse the molar mass of lignin, which can be a waste product in bioethanol production technology. A studies of isolated lignin from two species: *Populus deltoides x maximowiczii* (hardwood) and *Pinus sylvestris* L. (softwood) were conducted to determine its molar mass using the SEC technique. Two acidic methods of lignin isolation were used during the studies. The use of 72% sulphuric acid yielded higher values of M_n , M_w and PDI and the lignin was better dissolved in 0.5% LiCl/DMAc system, which determined the continued use of this method in further studies. The Klason lignin samples for both species were subjected to milling at three time configurations: 5, 15 and 30 min, which resulted in an increase in the aforementioned values. The tests carried out and the analysis of the results indicated that milling times longer than 5 min caused degradation and repolymerisation of the lignin, as confirmed by the molar mass distributions. Some of the wood samples were pretreated with steam explosion and next isolated Klason lignin were milled. SEC analysis showed a decrease in M_n values with increasing milling time, while an increase in M_w and PDI for steam exploded poplar lignin. For analogical obtained steam exploded pine lignin, the values increased directly proportional. For the milling carried out, 5 min was recommended, so that lignin solubility increased and molar mass was determined more reliable.

Keywords: poplar and pine wood, lignin, milling, SEC, steam explosion, bioethanol

INTRODUCTION

Long-term use of fossil fuels as the primary energy resource has been increasingly recognized as environmentally harmful for several years. Consequently, there is growing interest in seeking and developing alternative energy sources at the expense of reducing the high share of these conventional energy sources. Unstable geopolitical situations, rising oil prices, high greenhouse gas emissions, and a focus on ecological sustainability further drive the acceleration of these processes. Additionally, the European Union's policy and the introduction of new law regulations aiming to increase the consumption of renewable energy to 42.5% by 2030 make this topic even more significant.

In terms of the development of renewable energy sources, biofuel production is one of the main developments. Increasing the share of low-energy-intensity fuels in the overall energy share can effectively counteract climate changes [Abas et al. 2015]. The aforementioned process can be seen as a solution to the problems of energy security, energy feedstock demand and climate changes. However, the implementation of innovative technologies related to the biofuels production is associated with high investments [Broda et al. 2022]. Biofuels are products that ultimately aim to replace traditional fuels, and one of the leading precursors of this trend is bioethanol. It can be produced from renewable materials (lignocellulosic biomass) of various types, which have in common a rich content of carbohydrates [Broda et al. 2022]. A significant amount of these can result in hydrolysis to fermentable sugars and subsequent conversion to ethanol.

Lignocellulosic biomass is cell wall matter, which consists of three main polymers, i.e. cellulose (35-50%), hemicelluloses (20-32%) and lignin (10-35%) [Kumar et al. 2009], and may also contain trace amounts of pectin, nitrogenous compounds and inorganic components [Mori et al. 2015]. Its sources can be energy crops, forest resources, urban waste and residues obtained from mills, forest clearings and farms [Sun and Sun 2015]. Wood species with high potential for biomass production include willow (*Salix* sp.) and poplar (*Populus* sp.), which was one of the species selected for the study. Pine wood can also be taken into account due to its common occurrence in Poland and around the world, especially in the temperate climate zone of the northern hemisphere.

Lignin, a component of lignocellulosic biomass, that is the focus of this article, is one of the most abundant biopolymers in the world and accounts from 18% to 35% of the wood mass [Kumar et al. 2009]. In the wood of softwood species its percentage content (25-35%) is higher than for hardwood species (18-25%). Lignin is characterised by an amorphous structure, a highly branched and crosslinked three-dimensional polymer with no regularly repeating elements [Broda et al. 2022]. According to Klason and Fraudenberg, lignin is a condensation polymer whose basic radical is a phenylpropane skeleton where the aliphatic fragment can be a glycerin, hydroxyaldehyde or hydroxyketone radical [Krutul 2002]. In addition to aliphatic fragments, aromatic fragments can be distinguished in the structure of lignin, such as: guaiacyl, syringyl and p-hydroxyphenyl. The cellulose, hemicelluloses and lignin in wood are strongly linked by hydrogen bonds, van der Waals interactions and covalent bonds, which form a structurally compact composite matrix. According to Broda et al. [2022], lignin has a high resistance to depolymerisation and a low tendency to isolate. An excellent summary as to the nature of lignin is that it is a substance whose exact structure has not yet been fully defined.

In order to approach a understanding of the structure of lignin, its molar mass can be determined, the value of which determines its physicochemical properties and reactivity for value-added applications [Tolbert et al. 2014]. Knowledge of the molar mass of lignin can be used in industry to replace petrochemical-based aromatic compounds with lignin from biomass [El Mansouri and Salvadó 2006]. To do so, it is necessary to determine the number average molar mass (M_n), the weight average molar mass (M_w) and the polydispersity index (PDI). Typical molar masses of isolated lignin from plant biomass are in the range 1 000-20 000 g/mol [Doherty et al. 2011]. One of the best methods for determining the molar masses of polymers is size exclusion chromatography (SEC). Using this method, it is also possible to determine the molar mass distribution (MMD) of various polymers, including lignin.

In order to determine the molar mass of lignin, it must be previously isolated from native biomass. The literature mentions the use of different methods, but the most frequently are milled wood lignin (MWL), cellulolytic enzyme lignin (CEL) and enzymatic mild acidolysis lignin (EMAL) [Tolbert et al. 2014]. Tolbert et al. [2014] stated that for M_w of isolated lignin general trend is observed: EMAL > CEL > MWL. Another frequently used direct method for determining the lignin content is the TAPPI method [TAPPI T222 om-02 2006], in which the isolated lignin is called Klason lignin. This method is often recommended, due to its repeatability and reliability [Klason 1983; Krutul 2002; Dhaka et al. 2019]. It is a method where the yield and composition of isolated lignin depend on the concentration of sulphuric acid used and the treatment time and temperature [Dence 1992]. Too low a concentration of acid can lead to incomplete hydrolysis of the carbohydrates, while concentrations above 80% can lead to conversion of the carbohydrates into partially insoluble degradation products [Lai and Sarkanen 1971]. Unfortunately, under acidic conditions, condensation and repolymerization of lignin may occur, which may be unfavorable in terms

of its solubility and processability towards obtaining valuable substances [Shuai et al. 2016; Li et al. 2018; Lan and Luterbacher 2019].

Pretreatment of the material is often used in biomass processing. Pretreatment methods can be divided into four categories: physical, chemical, physico-chemical and biological [Broda et al. 2022]. They can reduce the crystallinity of cellulose, its polymerization degree, remove hemicelluloses, lignin, increase biomass specific surface area and facilitate its further processing [Tayyab et al. 2017]. Physical pretreatment processes such as chipping, coarse size reduction or milling are used to increase the digestibility of lignocellulosic biomass [Laser et al. 2002]. The introduction of milling prior to initial processing can lead to low energy consumption and no production of fermentation inhibitors [Tayyab et al. 2017]. Another benefit is also the reduction of solids-liquid separation costs by reducing the solids-liquid ratio [Zhu et al. 2010]. Disadvantages of this method include a higher energy requirement compared to other methods [Hideno et al. 2009] however, Cadoche and Lopez [1989] mention that this is dependent on the characteristics of the agricultural mass and the final particle size.

Physico-chemical pretreatments are a combination of mechanical and chemical processes [Tayyab et al. 2017]. An examples of such treatments are the steam explosion (SE), liquid hot water (LHW), soaking aqueous ammonia (SAA) or treatment with supercritical carbon dioxide at elevated temperatures and pressures on lignocellulosic biomass. The aforementioned SE consists of feedstock heating with steam contribution at high temperatures and pressures, followed by sudden pressure reduction and biomass explosive decompression. The process results in a material where the individual components can be obtained more easily than before the procedure. On the other hand, Michalska [2020] mentioned that the steam explosion method was more effective for hardwood, while softwood was not as susceptible to this treatment due to its high lignin content. According to Grous et al. [1986], pretreating poplar chips with superheated steam can increase the efficiency of the enzymatic hydrolysis process up to 90%, where the value without pretreatment was only 15%.

According to the various literature studies carried out, there is still a problem for determining the actual molar mass value of lignin and the available literature does not fully address the issues presented. Therefore, an attempt was made to carry out a study on isolated lignin from two tree species (hardwood and softwood) so as to determine its molar mass using the SEC technique. The studies aimed the analysis of lignin molar mass which can produce as waste in bioethanol production technology.

MATERIAL AND METHODS

Material characteristic

Maximowicz poplar (*Populus deltoides* x *maximowiczii*) and Scots pine (*Pinus sylvestris* L.) wood were used for the study. The poplar wood came from experimental field located in Wolica, owned by the Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology at Warsaw University of Life Sciences. The age of the poplar tree was 7 years. The pine wood, on the other hand, came from the Białowieża Forest District, located in the Białowieża Forest in the Podlaskie Voivodeship. The studied material (a mixture of sapwood and heartwood) was collected from 30 years old pine tree. For both species, the material was obtained from previously debarked trunks, milled, and sorted to obtain the desired fraction from 0.43mm to 1.02mm.

At the beginning, native wood was dried to achieve a constant weight in a laboratory dryer at a temperature of 103 ± 2 °C. Then, extraction of the material was performed according to the method proposed by Krutul [2002] using a Soxhlet apparatus with a mixture of chloroform and ethanol in a weight ratio of 93:7 [Antczak et al. 2006]. The extraction time was 10 hours.

The extracted material was used to content determination of wood structural substances. Mineral substances content was determined in the form of ash on native wood using muffle furnace [Sluiter et al. 2008]. The chemical composition of studied wood was presented in Table 1.

Table 1. Chemical composition of studied poplar and pine wood

Component [%]	<i>Populus deltoides</i> <i>x maximowiczii</i>	<i>Pinus sylvestris</i> L.	Method source
extractives	2.1 ± 0.0	2.0 ± 0.0	chloroform-ethanol mixture (93:7) _{w/w} [Antczak et al. 2006]
Klason lignin (H ₂ SO ₄)	18.4 ± 0.4	28.6 ± 0.4	TAPPI T222 om-02 2006
lignin filtrate	2.1 ± 0.1	0.4 ± 0.0	TAPPI UM 250 1985
lignin (H ₂ SO ₄ + H ₃ PO ₄)	19.5 ± 0.4	29.2 ± 0.4	Krutul 2002
cellulose	50.4 ± 0.4	56.5 ± 0.4	Kürschner-Hoffer [Saeman et al. 1954; Krutul 2002]
holocellulose	83.1 ± 0.2	75.5 ± 0.4	sodium chlorite [Wise et al. 1946]
hemicelluloses	32.7	19.0	calculated as the difference between the holocellulose and cellulose contents
mineral substances	2.1 ± 0.0	2.0 ± 0.0	in the ash form [Sluiter et al. 2008]

Steam explosion pretreatment of wood

The steam explosion pretreatment was conducted on poplar and pine wood (both about 20 g, fraction from 0.43mm to 1.02mm) as a part of earlier studies [Michalska 2020]. The SE procedure was described in detail in a previous publication [Antczak et al. 2022]. The pretreatment process was carried out at 190 °C for 15 minutes. The pressure inside the reactor was approximately 12 bar. The unwashed solid fraction obtained as a result of SE pretreatment was stored in a refrigerator at 6 °C until tests related to lignin isolation and size exclusion chromatography analysis were performed. Two repetitions of SE treatment were performed for each wood species.

Lignin isolation

The lignin was isolated directly from poplar and pine dried extracted wood and also from dried extracted solid fraction obtained by SE pretreatment. To material extraction a mixture of chloroform and ethanol in a weight ratio of 93:7 was used [Antczak et al. 2006]. Time of extraction for wood was 10 hours and for solid fraction was 15 hours. Lignin from wood was isolated according to TAPPI method utilizing 72% sulfuric acid VI (H₂SO₄) [TAPPI T222 om-02 2006] and method described by Krutul [2002] using a mixture of 75% sulfuric acid VI and 89% orthophosphoric acid. Whereas lignin from solid fraction obtained after SE treatment was isolated only by one method - TAPPI method using 72% H₂SO₄.

Finally, the isolated samples of lignin were washed with distilled water to pH=6 and dried to achieve a constant weight at a temperature of 103 ± 2 °C.

Lignin pretreatment - milling

In order to enhance a lignin solubility, before SEC analysis, a part of lignin samples (directly isolated from wood and from SE solid fraction by TAPPI method) were milled using a ball mill. The milling time was 5, 15 and 30 min and the frequency was 30 Hz.

Dissolution of lignin samples for SEC analysis

The prepared lignin samples were dissolved in order to perform SEC analysis and determine the molar mass of lignin. The lignin samples, which were dissolved for SEC analysis were presented in Table 2.

Table 2. The lignin samples dissolved for SEC analysis

Lignin sample	Poplar wood	Pine wood	SE solid fraction from poplar wood	SE solid fraction from pine wood
Klason lignin (H ₂ SO ₄)	+	+	-	-
lignin (H ₂ SO ₄ + H ₃ PO ₄)	+	+	-	-
Klason lignin (H ₂ SO ₄); milled for 5, 15 and 30 min	+	+	+	+

The dissolution procedure was as follows:

- 10 mg each of lignin sample was weighted in a glass screw-cup tube with a volume of 10 cm³;
- then 5 cm³ of 0.5% LiCl/DMAc was added and the test tubes were screwed;
- the lignin dissolution was realized using rotary mixer (RM-2M, Elmi company);
- after 3 weeks of dissolution, samples were filtered using 0.22 µm Nylon syringe filter;
- finally, prepared samples were submitted to SEC analysis. For each sample, three analyses were performed.

Conditions of SEC analysis

SEC analysis of lignin samples was performed using the HPLC (High Performance Liquid Chromatography) system (LC-20AD, Shimadzu company), which was equipped with a differential refractive detector (RID 10A, Shimadzu), pump (LC-20AD, Shimadzu), and oven (CTO-20A, Shimadzu). SEC analysis conditions were as follows:

- 0.5% LiCl/DMAc as eluent
- two connected columns filled with crosslinked polystyrene-divinylbenzene gel (MIXED-B, 10 µ, 7.5×300 mm) and PLgel guard column (GUARD, 10 µ, 7.5×50 mm)
- oven temperature: 80 °C
- flow rate of the eluent: 1 cm³/min
- injection volume of sample: 0.2 cm³.

The chromatographic data were processed with PSS WinGPC scientific 2.74 software and PSS calibration program V2.99 (Polymer Standard Service, Mainz, Germany). Eight narrow molar mass polystyrene standards (Agilent Technologies, Palo Alto, USA) were used to calibrate the column (standard conventional calibration). The polystyrene standards were prepared as mixed standards in two separate solutions in 0.5% LiCl/DMAc. The first standard solution contained polystyrene of the following peak molar mass: 6 570 000, 729 500, 117 700 and 27 810 g/mol, and the second contained: 2 403 000, 301 600, 70 500 and 9 570 g/mol. These polystyrene standards were used for direct molar mass of lignin determination.

RESULTS AND DISCUSSION

In this section, the obtained results regarding the SEC analysis of lignin were presented and discussed. The results of the weight average and number average molar masses along with the polydispersity index for lignins from two types of wood obtained by two acidic methods were shown in Table 3. The aforementioned data after the milling process of Klason lignins in three time configurations for both types of wood were also presented.

Table 3. The M_n , M_w and PDI of lignin isolated from poplar and pine wood by acids methods

Lignin sample	M_n [g/mol]	M_w [g/mol]	PDI
Poplar wood			
Klason lignin (H ₂ SO ₄)	8 339 ± 233	53 577 ± 2 251	6.4 ± 0.3
lignin (H ₂ SO ₄ + H ₃ PO ₄)	4 126 ± 443	8 530 ± 618	2.1 ± 0.1
Klason lignin (H ₂ SO ₄); milled for 5 min	8 657 ± 155	57 765 ± 2 263	6.7 ± 0.2
Klason lignin (H ₂ SO ₄); milled for 15 min	8 810 ± 279	68 830 ± 1 666	7.8 ± 0.2
Klason lignin (H ₂ SO ₄); milled for 30 min	10 545 ± 695	102 753 ± 6 391	9.8 ± 0.5
Pine wood			
Klason lignin (H ₂ SO ₄)	4 653 ± 824	6 092 ± 358	1.3 ± 0.3
lignin (H ₂ SO ₄ + H ₃ PO ₄)	2 278 ± 337	3 513 ± 425	1.5 ± 0.0
Klason lignin (H ₂ SO ₄); milled for 5 min	5 069 ± 219	13 883 ± 387	2.7 ± 0.2
Klason lignin (H ₂ SO ₄); milled for 15 min	6 488 ± 162	25 647 ± 1 901	3.9 ± 0.2
Klason lignin (H ₂ SO ₄); milled for 30 min	7 628 ± 173	31 067 ± 1 898	4.1 ± 0.2

Comparing the results for lignin obtained by two acidic methods, poplar lignin had higher values of M_n , M_w and PDI than lignin from pine wood. Analyzing these results in terms of the lignin isolation method, using 72% sulfuric acid, the results for both species were higher than those obtained using the method with mixture of sulfuric and orthophosphoric acids. For Klason lignin (without milling) isolated from poplar wood, the M_w value was 53 577 g/mol, but for lignin isolated from this wood by mixture of H₂SO₄ and H₃PO₄ the M_w value was 8 530 g/mol only. Also, the M_n , M_w and PDI values obtained for lignin by other researchers are different, depending on method isolation or analysis and vary within a wide range. For example, Baumberger et al. [2002] found, that for lignin isolated from *Populus deltoides* x *populus nigra* wood, the M_w value was 36 000 g/mol, M_n was 8 700 g/mol and PDI was 4.1. However, the values provided by Moon et al. [2011] for lignin from *Populus alba* x *populus glandulosa* wood were 13 250 g/mol, 4 176 g/mol and 3.2, respectively.

In the case of lignin derived from pine wood, a similar trend was observed: the M_w for Klason lignin (6 092 g/mol) was higher than M_w for lignin (3 513 g/mol) isolated by mixture of H_2SO_4 and H_3PO_4 . According to Guerra et al. [2006], the values of M_w , M_n and PDI for lignin from *Pinus palustris* Mill. wood were 14 900 g/mol, 4 700 g/mol and 3.2, respectively. In turn, Sannigrahi et al. [2010] found that lignin isolated from *Pinus taeda* L. wood was characterized by the following values: 13 500 g/mol, 7 590 g/mol and 1.8, respectively.

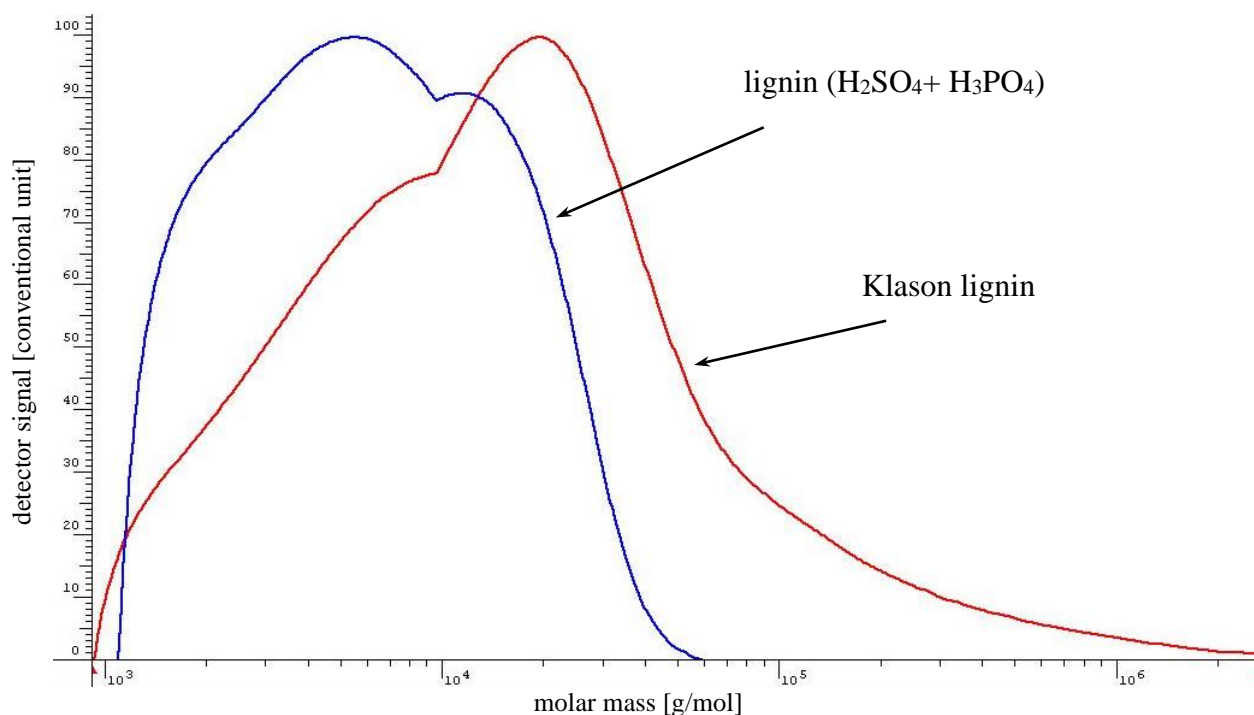


Fig. 1. Molar mass distributions of lignin isolated from *Populus deltoides x maximowiczii* wood by acids methods

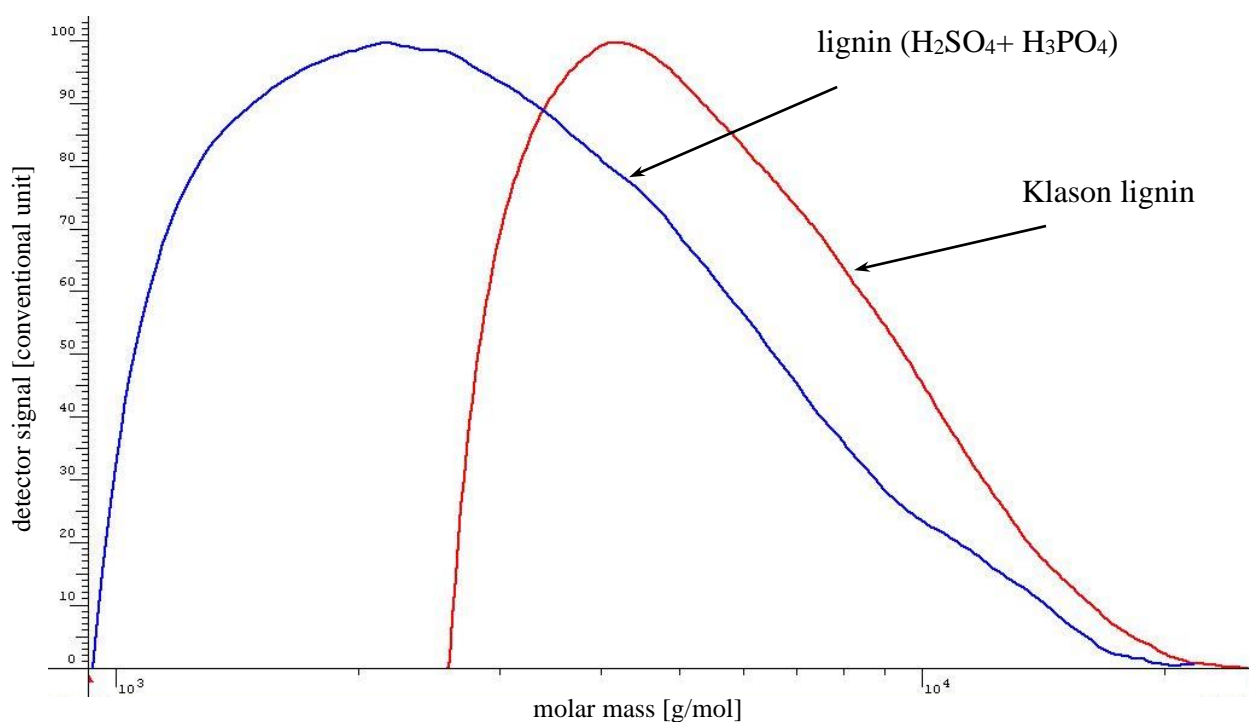


Fig. 2. Molar mass distributions of lignin isolated from *Pinus sylvestris* L. wood by acids methods

According to the authors of this work, the results from the SEC analysis of lignin isolated using 72% sulfuric acid for poplar and pine wood are more reliable, because the lignin samples were better dissolved. This is confirmed by the molar mass distributions shown in Figs 1 and 2. The MMDs of Klason lignin were broad and covered both the low and high molar mass lignin fractions. Hence, Klason lignin was selected for further studies related to pretreatment. However, according to Yuan et al. [2010], the lignin obtained in acidic conditions may have a highly condensed structure therefore, in further processes, milling was applied for three different durations: 5, 15, and 30 minutes, aiming to facilitate the solubility of lignin during the dissolution process in LiCl/DMAc system.

If we consider the impact of the milling process on lignin parameters studied by SEC technique, a gradual increase in M_n , M_w and PDI can be observed (Table 3). In case of poplar lignin, M_w after 5 minutes of milling was 57 765 g/mol, after 15 minutes was 68 830 g/mol, while after 30 minutes, it was already 102 753 g/mol. For lignin derived from pine wood, similar trends can be observed. The M_w of pine lignin after 30 minutes of milling increased from 6 092 to 31 067 g/mol.

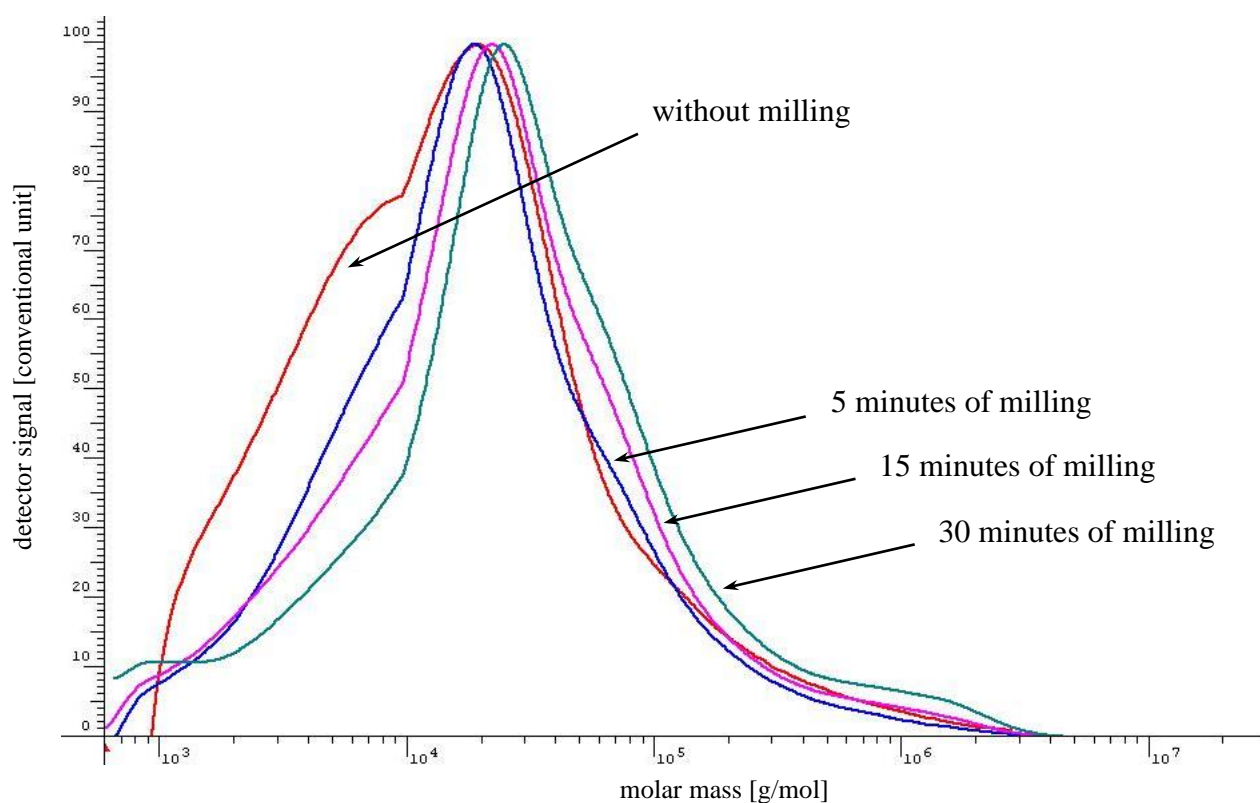


Fig. 3. Molar mass distributions of Klason lignin isolated from *Populus deltoides* x *maximowiczii* wood without milling and after different times of milling

The gradual increase of these parameters may indicate better lignin solubility on the one hand, and on the other simultaneous degradation and repolymerization reactions of the lignin structures, which is an undesirable phenomenon. This was confirmed by the molar mass distributions shown in Figs 3 and 4. As a result of milling, a gradual shift in distributions towards higher molar masses can be observed, which indicates the processes of condensation and repolymerization of lignin structures. However, the least increase of M_w was recorded for 5 minutes of milling, suggesting that this duration should be considered in further studies. This may be especially important for lignin from pine wood, as the short-term

milling process had a beneficial effect on its solubility what was reflected on MMD (Fig. 4). The molar mass distribution curve of pine lignin after 5 minutes of milling is much wider than the MMD curve without milling. This indicates better solubility of pine lignin, as both low and high molar mass lignin fractions were dissolved.

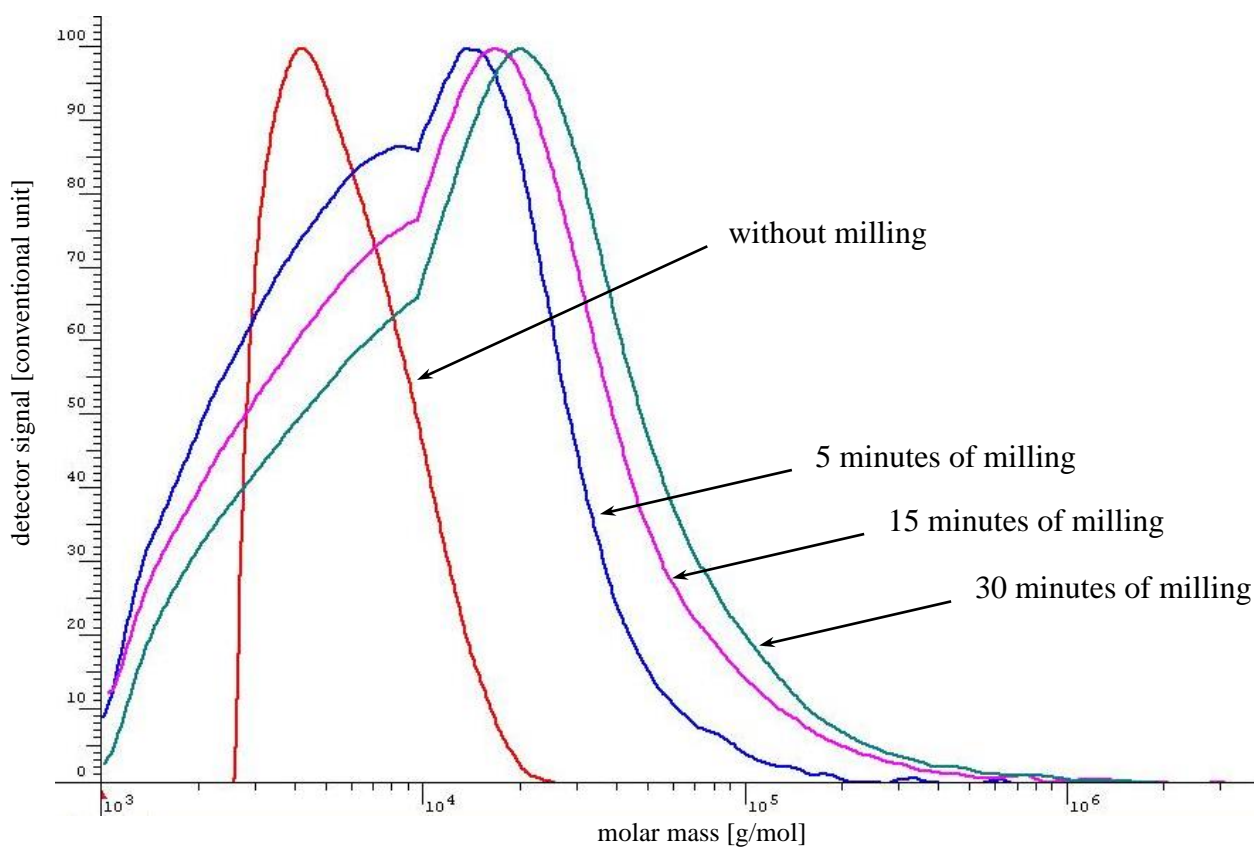


Fig. 4. Molar mass distributions of Klason lignin isolated from *Pinus sylvestris* L. wood without milling and after different times of milling

In Table 4 the values of M_n , M_w and PDI of Klason lignin isolated from solid fraction obtained by steam explosion pretreatment of poplar and pine wood were presented. Additionally, isolated Klason lignin was milled in three time configurations (5, 15 and 30 minutes). Analyzing the results presented in Table 4, the values for Klason lignin obtained from SE poplar solid fraction showed a noticeable decrease of M_n values proportional to the increase of milling time. In contrast, the M_w values increased accordingly, as did the PDI index. In this case, the processes of lignin repolymerization and condensation dominated. According to data obtained by other researchers [Baumberger et al. 2007] for lignin from *Populus tremula* L. wood subjected to steam explosion, the M_w value was 34 590 g/mol and M_n value was 2 180 g/mol. Comparing these results with those in Table 4 for poplar lignin, the M_w value is closest to Klason lignin subjected to 5 minutes of milling, while Klason lignin milled for 5 minutes had a higher M_n value than compared literature data. In the case of lignin obtained from SE pine solid fraction, an increase in all analyzed parameters was noted relative to the increase of milling time. Considering the currently available researches, no appropriate data was found to which the obtained results could be referenced.

The impact of steam explosion on the lignin isolated from poplar solid fraction resulted in decrease of M_w compared to values for lignin isolated from native poplar wood (Tables 3 and 4). In the case of lignin obtained from pine SE solid fraction, the values of the analyzed

weight average molar masses increased in comparison to M_w of lignin obtained from native wood (Tables 3 and 4).

Table 4. The M_n , M_w and PDI of Klason lignin isolated from solid fraction obtained by SE pretreatment of poplar and pine wood

Lignin sample	M_n [g/mol]	M_w [g/mol]	PDI
SE solid fraction from poplar wood			
Klason lignin (H ₂ SO ₄); milled for 5 min	6 473 ± 934	29 340 ± 2 702	4.6 ± 0.6
Klason lignin (H ₂ SO ₄); milled for 15 min	4 835 ± 454	63 927 ± 3 921	13.3 ± 0.7
Klason lignin (H ₂ SO ₄); milled for 30 min	2 623 ± 80	70 670 ± 2 063	26.9 ± 0.1
SE solid fraction from pine wood			
Klason lignin (H ₂ SO ₄); milled for 5 min	5 557 ± 281	21 117 ± 2 803	3.8 ± 0.5
Klason lignin (H ₂ SO ₄); milled for 15 min	7 571 ± 733	31 210 ± 1 895	4.1 ± 0.1
Klason lignin (H ₂ SO ₄); milled for 30 min	7 672 ± 622	43 717 ± 1 522	5.7 ± 0.4

These relationships are consistent with the observations of other researchers [Wang et al. 2020] who also found that as a result of steam explosion pretreatment, depolymerization and repolymerization of lignin occurred, which influenced its structure and weight average molar mass. According to analyses conducted by Ramos [2003], higher values of the analyzed M_w may result from the fact that hardwood lignin contains a higher percentage of methoxyl groups, is less condensed and more susceptible to dissolution or chemical conversion compared to softwood lignin. This assertion may explain the higher weight average molar masses for poplar lignin compared to pine lignin (Tables 3 and 4). The pretreatment used in this work in the form of short-term milling (up to 5 minutes) enabled better dissolution of lignin and a more reliable determination of its molar mass by SEC method.

SUMMARY AND CONCLUSIONS

On the basis of the studies performed, the following observations and conclusions were drawn:

1. The M_n , M_w and PDI values obtained for lignin by SEC technique were different, depending on method isolation, wood species and varied within a wide range. For poplar lignin isolated by acids methods these parameters were higher than for pine lignin.
2. The method using 72% sulfuric acid for lignin isolation resulted in obtaining higher M_n , M_w and PDI values for both wood species than using a mixture of sulfuric and orthophosphoric acids. The samples of Klason lignin were better dissolved and the SEC results more reliable.
3. Short-term lignin milling (up to 5 minutes) was beneficial, because a lignin samples were better dissolved in 0.5% LiCl/DMAc system, which allowed for a more reliable determination of molar mass using the SEC method. This was especially important for pine lignin, which is generally more condensed and less susceptible to dissolution.

4. Longer milling times led to lignin degradation and repolymerization, which was associated with an increase in M_n , M_w and PDI values, as confirmed by MMD distributions.
5. In relation to lignin isolated from native wood, the steam explosion of poplar lignin caused decrease of M_w values, while in the case of pine lignin, an inverse relationship was observed. This is related to lignin degradation and repolymerization phenomena.

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Streszczenie: Analiza SEC masy cząsteczkowej ligniny wyodrębnionej z drewna topoli (*Populus deltoides x maximowiczii*) i sosny zwyczajnej (*Pinus sylvestris* L.). Celem badań była analiza masy cząsteczkowej ligniny, która może stanowić produkt odpadowy w technologii produkcji bioetanolu. Przeprowadzono badania wyodrębnionej ligniny z dwóch gatunków: *Populus deltoides x maximowiczii* (drewno liściaste) i *Pinus sylvestris* L. (drewno iglaste) w celu określenia jej masy cząsteczkowej techniką SEC. W badaniach zastosowano dwie kwasowe metody wyodrębniania ligniny. Zastosowanie 72% kwasu siarkowego pozwoliło uzyskać wyższe wartości M_n , M_w i PDI, a lignina lepiej rozpuszczała się w układzie 0,5% LiCl/DMAc, co determinowało kontynuację stosowania tej metody w dalszych badaniach. Próbkę ligniny Klasona obu gatunków poddano mieleniu w trzech konfiguracjach czasowych: 5, 15 i 30 min, co spowodowało wzrost ww. wartości. Przeprowadzone badania i analiza wyników wykazały, że czasy mielenia dłuższe niż 5 min powodują degradację i repolimeryzację ligniny, co potwierdzają rozkłady mas cząsteczkowych. Część próbek drewna poddano wstępnej obróbce wybuchem pary, a następnie wyodrębnioną ligninę Klasona zmielono. Analiza SEC wykazała spadek wartości M_n wraz ze wzrostem czasu mielenia oraz wzrost M_w i PDI dla ligniny topolowej poddanej obróbce wstępnej wybuchem pary. Dla analogicznie otrzymanej ligniny sosnowej poddanej obróbce wstępnej wybuchem pary wartości wzrosły wprost proporcjonalnie. Dla przeprowadzonego mielenia zalecano czas 5 min i w ten sposób zwiększono rozpuszczalność ligniny, co umożliwiło w sposób bardziej wiarygodny określić jej masę cząsteczkową.

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