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A SELECTION OF THE APPLICATION OF *PINUS SYLVESTRIS* L. FROM PUSZCZA NOTECKA BY CHEMICAL ANALYSIS

Pinus sylvestris L. is the most popular wood material used in building construction and pulp technology. However, it can be also applied for other, more economically beneficial purposes like ethanol, commercial quantities of xylose or glucose as well as substrates for chemical synthesis. The selection of an optimal conversion path of wood should be based on its chemical composition and physical properties. The overall aim of this research was to determine the chemical composition and features of cellulose and lignin structure on a molecular level of Pinus sylvestris L. wood from a primeval forest: Puszcza Notecka was chosen because of its valorisation. The trees from four stands: two from the primeval forest and for comparison two from stands out of the primeval forest were used in the research. The percentage of chemical components of the wood: holocellulose, cellulose, pentosans and lignin as well as components soluble in ethanol and ash were determined. The chemical analysis provided information on the number of wood components. Their variations at a molecular level were investigated by Py-GC/MS, highlighting how the growth place of trees can affect the formation of wood pyrolysis products. The differences between the structures of cellulose and lignin, amidst certain feedstocks were also analysed by FTIR.

It was shown that a percentage of the chemical composition of material from the stands in the primeval forest is more homogeneous in comparison to material from other investigated stands.

There was a lower content of extractives in wood from the primeval forest which facilitates the gluing and finishing of wood-based materials. A low content of ash in this material gives possibilities to consider using investigated feedstock for energy purposes. The highest content of holocellulose, 77.5%, was found in the wood gained from the primeval forest and this feedstock is the best for obtaining carbohydrate derivatives. Both the low content of lignin and the low content of extractives in wood from Puszcza Notecka allowed them to be applied

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in the fermentation process. The evaluation of the relative amounts of pyrolysis products deriving from holocellulose and lignin and FTIR analysis highlighted differences between feedstock growing in the compared areas. The results obtained indicated that the trees from Puszcza Notecka are an attractive feedstock for technological branches due to the homogeneous chemical and physical features and they can potentially be used for countless economically-viable applications.

Keywords: softwood, wood valorisation, wood quality, chemical composition

Introduction

Scots pine is a basic species in Polish forests, covering 69.2% of the volume in the State Forests [The National Forest Inventory 2015]. Its application ranges from pulp production, the furniture industry, the production of chipboard, fibreboard, veneers, plywood and to artistic woodwork. Wood, wool and charcoal are produced from it. This species is also used as a construction material for producing windows, doors, floors, railway sleepers, masts and stanchions. According to the studies of Korica et al. [2015] it's possible to produce pine essential oils throughout the year and the product corresponds to the standards for an industrial product. A key factor for increasing profit in the wood industry is to increase the feedstock utilisation. One of the methods is to rationally manage wood waste produced during felling, mechanical or chemical treatment [Kibblewhite 1984]. It is justifiable to search for an increase of product value obtained from a unit of a purchased feedstock, therefore, it's significant to improve the quality of wood in order to increase its value [Macdonald et al. 2010].

The primeval forest Puszcza Notecka is one of the biggest forest complexes in Poland covering approx. 135 000 ha. It grows on dunes between the Warta and the Noteć rivers in the Kotlina Gorzowska region. It comprises of mainly dense stands of *Pinus sylvestris* L. with small amounts of other forest species such as *Betula pendula* Roth., *Alnus glutinosa* Gaertn., *Fagus sylvatica* L., *Quercus robur* L., *Quercus petraea* (Matt.) Liebl. and *Picea abies* (L.) H. Karst. In 2004 'The Forest Promotion Complex' "Puszcza Notecka" was established to assist in conducting balanced forest economy, educating society on nature-forest issues, developing scientific research as well as implementing new technologies. Currently, primeval forest stands comprise of intensively utilised mature trees bringing 800-900 thousand m³ of wood annually and that number is growing. About 70% of the Scots pine roundwood from Puszcza Notecka is utilised in the cellulose technology and wood-based materials production whereas only small quantities of it are used in higher value markets such as pharmaceutical, biotechnology or the chemical industry. It is important to evaluate the quality of timber to maximise the value of Scots pine resources. Bowyer [2016] highlights that nowadays the most important financial opportunities for the forest sector are

amongst others, innovative applications of wood in creating commercial structures, including high buildings, using wood as a feedstock in industrial chemical production and in the nanotechnology industry and finally as wood energy, including wood pellets and wood derived biofuels.

Increasing industry's wood biomass demand for energy purposes results in the recognition of the content and structure of the lignocellulosic components before making a decision on the designation of a feedstock to the industry sector [Sandak and Sandak 2011; Sable et al. 2012; Xu et al. 2013; Zborowska et al. 2013].

Investigations related to the chemical composition of softwood relating to the habitat were performed earlier for different areas of Europe [Sandak et al. 2012; Fernandes et al. 2017; Sensuła et al. 2017]. Recognition of the favourable wood properties from Puszcza Notecka (Poland) has encouraged the investigation of its physical properties in order to make full use of its advantages [Roszyk et al. 2016]. In this paper, we present the results of the chemical investigation of Scots pine timber growing in Puszcza Notecka which can provide a sufficient quantity of feedstock to operate on a multiproduct basis.

Materials and methods

In the investigation, *Pinus sylvestris* L. from the Forest Inspectorate Międzychód was used. Two stands in the primeval forest Puszcza Notecka were selected: stand 1 in Sowa Góra and stand 2 in Zamyślin. For comparison, two stands outside of the primeval forest were chosen: stand 3 in a forest growing on a post-agricultural area in Muchocin and stand 4 in a forest in Goraj (fig.1). In all the researched stands the forest type was fresh forest, whereas the commercial stand was Scots pine (100% of the stand). The age of the trees was estimated to be 80 years. Three trees typical for each stand were selected for the research of chemical properties. In total, 12 trees were investigated.

The slices of Scots pine wood were conditioned at a temperature of $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and RH of $60 \pm 3\%$ to obtain 12% of moisture. Afterwards, the samples were ground with a circular saw, clippers and finally with a knife mill Pulverisette 15. The final stage of preparing the material for chemical analysis was conditioning the wood to 8% moisture and obtaining an analytical fraction of 0.1-0.4 mm, according to PN-86/M-04001. The percentage content of holocellulose, cellulose, lignin and extractives (with 96% ethanol) and minerals were determined according to PN-92/P-50092. The pentosans were determined under the methodology given by Prosiński [1984] taking into account the precipitation of furfural with phloroglucinol. The presented results are an average of the three analyses.

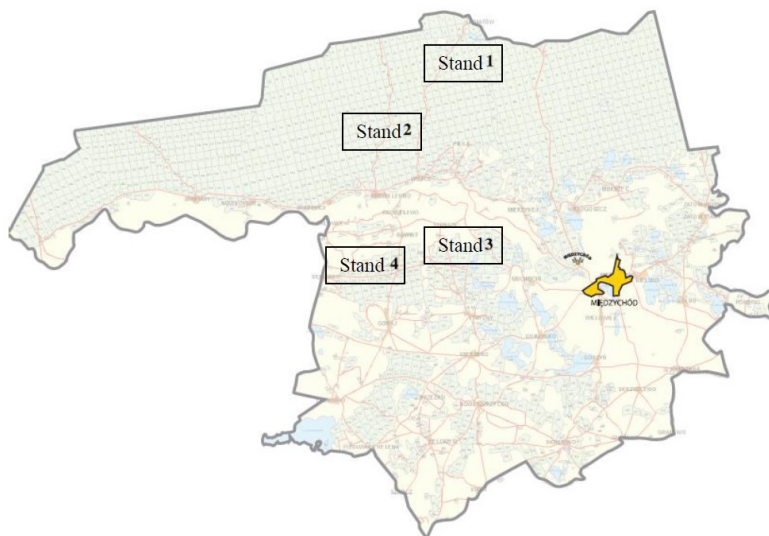


Fig. 1. Location of trees growth used in studies: 1 – Sowia Góra, 2 – Zamyślin, 3 – Muchocin, 4 – Goraj

Changes in cellulose were evaluated based on the total crystallinity index and lateral order index. Fourier Transform-Infrared (FT-IR) spectroscopy was used to determine both cellulose crystallinity indices. FT-IR spectra were obtained by means of an Alfa FT-IR spectrometer (Bruker Optics GmbH, Germany). Powder samples of the cellulose (2 mg) were dispersed in a matrix of KBr (200 mg), followed by compression to form pellets. The sample collection was obtained using 32 scans, ranging from 4000 to 400 cm^{-1} , at a resolution of 4 cm^{-1} . Three measurements for each cellulose sample were made, and the average value was considered. The total Crystallinity Index (TCI, A1370/A2900) and Lateral Order Index (LOI, A1430/A898) were calculated according to Nelson and O'Connor [1964]. The structure of lignin (Al/Ar A2930/A1510 and C=O A1710/A1510) was determined according to Ungureanu et al. [2009].

An analytical pyrolysis was performed using a CDS Pyroprobe 5000 Series. The sample (100 μg) was placed in a quartz tube, 2 mm \times 4 mm. The pyrolysis was carried out at 550°C for 20 s. The pyrolyser was connected to a TRACE 1300 gas chromatograph equipped with a fused silica capillary column (007-624, 30 m \times 0.25 mm ID, 1.5 μm film thickness) coupled to an ISQ Single Quadrupole mass spectrometer. The chromatograph was programmed to 40°C (5 min), 10°C min^{-1} to 240°C, 20°C min^{-1} to 300°C. The final temperature was held for 5 min. The injector and the GC/MS interface were set at 200°C. The compounds were identified by comparing the mass spectra thus obtained with those of the Wiley computer libraries. Quantification was based on peak areas.

The areas were normalized for each chromatogram, and the data from the analyses were expressed as percentages.

The statistical analysis was done using STATISTICA 10 and consistent with ANOVA followed by a post hoc Tukey's test. The identical letters in certain columns represent no differences at the significance level $p = 0.05$.

Results and discussion

The results of the determination of minor components *i.e.* extractives and ash for three trees from each stand: 1-4 and the average for a given stand are presented in table 1. Extractives are complex components including different constituencies typical for a given wood species. In the chemical composition of *Pinus sylvestris* L. pinosylvin, pinosylvin monomethyl ether, resin acids and free fatty acids were identified among the others [Ekeberg et al. 2006]. Their concentration and distribution vary and depend on growing conditions, age and location in a radial direction and trunk height [Hovelstad et al. 2006; Tümen and Reunanen 2010]. The percentage of extractives in the investigated trees ranged from 2.5% to 5.9%. According to literature [Prosiński 1984], the content of substances in Scots pine which undergo extraction ranges from 3.2% to 5.9%. The highest extractives' differentiation in one stand and simultaneously the lowest values were stated for the trees from the primeval forest in Sowie Góra. The average values gained for all the investigated stands ranged from 4.2% to 5.1%. According to Hse and Kuo [1988], extractives change the wettability and the curing properties of adhesives. A desirable wettability-permeability relationship is sometimes affected by extractives, thus reducing the glue-bond strength and performance. The most common deleterious effect of extractives on finishing is the discoloration of coatings and paint films. Therefore, the material from Puszcza Notecka has better properties concerning gluing and finishing. Considering the possibilities of application of the researched feedstock for energy purposes it should be mentioned that according to White [1987] extractives increase the combustion value of lignocellulosic biomass. However, research connected with the application of natural extractives in firewood protection was conducted [Baysal et al. 2007]. According to the results of Lindberg et al. [2004] extractives decrease or inhibit bacterial growth. One of the components which is a fungitoxin protecting the wood from fungal infection is pinosylvin, hence having a potential application as a natural biocide in paper-making. Therefore, the materials from the outside of the primeval forest, which include more extractives, have potentially better microorganism resistance. Those materials also provide the highest amount of resin products such as rosin, terpenes and tall oil [Surmiński 1994].

Minerals play an important role in plant metabolism as they are components of organic structures, such as proteins and nucleic acids which are essential constituents of living organisms [Zule and Dolenc 2012]. The ash content in

wood tissue depends on numerous factors, such as growing conditions, wood age, place or time of taking samples. The percentage of ash in the investigated trees obtained from the four compared stands ranged from 0.2% to 0.3%. Those values do not vary from published data [Prosiński 1984] according to which, states that the content of ash in Scots pine is 0.2% to 0.5%. The slight discrepancy of the values may indicate the significant similarity of growing conditions of the tested trees. Minerals should be taken into account while considering energy purposes [Piechocki et al. 2014]. For example, the high content of chlorine is the reason for exploitation problems of boilers such as chloride corrosion [Mudgal et al. 2014]. Even if biomass does not contain chlorine, high levels of potassium in lignocellulose biomass ash cause increased slugging. Therefore, the values for the investigated trees give possibilities to consider this feedstock to be used for energy purposes.

Table 1. Percentage content of minor components of *Pinus sylvestris* L. from stands 1-4

Stand	Extractives [%]		Minerals [%]	Minerals [%]
	Three trees from each stand	Average for stand	Three trees from each stand	Average for stand
Primeval forest Sowia Góra/ 1	2.5 ^a ±0.2	4.2 ±0.1	0.3 ^a ±0.0	0.3 ±0.0
	4.7 ^b ±0.1		0.3 ^a ±0.0	
	5.4 ^c ±0.1		0.3 ^a ±0.0	
Primeval forest Zamyślin/ 2	4.8 ^a ±0.1	4.5 ±0.2	0.2 ^a ±0.0	0.2 ±0.0
	4.8 ^a ±0.4		0.2 ^a ±0.0	
	4.0 ^b ±0.2		0.3 ^b ±0.0	
Post-agricultural Muchocin/ 3	5.6 ^a ±0.2	5.1 ±0.2	0.2 ^a ±0.0	0.2 ±0.0
	5.6 ^a ±0.3		0.2 ^a ±0.0	
	4.0 ^b ±0.0		0.2 ^a ±0.0	
Forest Goraj/ 4	5.9 ^a ±0.2	5.0 ±0.2	0.2 ^a ±0.0	0.2 ±0.0
	4.1 ^b ±0.2		0.2 ^a ±0.0	
	5.0 ^c ±0.1		0.2 ^a ±0.0	

Mean values (n = 3) ± standard deviations; identical superscripts (a. b. c..) denote no significant (p < 0.05) difference between mean values in columns according to Tukey's HSD test (ANOVA) for investigated wood samples.

The components which influence wood strength properties and significantly determine the possibility of applying wood for a specified purpose are structural components such as carbohydrates and lignin. The results of the percentage of trees' main components obtained from the four stands (1-4) and average values for a given stand are presented in table 2. The trees from the four stands were characterised by a high content of holocellulose ranging from 74.5% to 80.0%, which is an indicator of the huge attractiveness of this feedstock for chemical processing. Higher inhomogeneity of the obtained results was recorded for the trees growing in stand 1 in Sowia Góra and stand 4 in Goraj. A homogeneous

material in terms of the content of holocellulose was growing in stands 2 in Zamyślin and 3 in Muchocin. The average content of holocellulose for each stand ranges from 74.2% to 77.5%. The highest content of holocellulose, 77.5%, was found in the trees gained from the primeval forest in Sowia Góra. The lowest content of this component – 74.2% was also found in the trees from the primeval forest in Zamyślin.

Table 2. Percentage content of main components of *Pinus sylvestris* L. from stands 1-4

Stand	Holocellulose [%]		Cellulose [%]		Pentosans [%]		Lignin [%]	Lignin [%]
	Three trees from each stand	Average for stand	Three trees from each stand	Average for stand	Three trees from each stand	Average for stand	Three trees from each stand	Average for stand
Primeval forest Sowia Góra/1	80.0 ^a ±0.1		47.5 ^a ±0.7		12.5 ^a ±0.1		27.6 ^a ±0.1	
	75.3 ^b ±0.4	77.5±2.4	46.6 ^a ±1.0	47.6±0.5	11.9 ^a ±0.3	12.3±0.3	27.8 ^a ±0.1	27.7±0.1
	76.6 ^b ±0.2		47.0 ^a ±0.4		12.2 ^a ±0.5		27.7 ^a ±0.2	
Primeval forest Zamyślin/2	75.6 ^a ±2.5		47.6 ^a ±1.4		11.5 ^b ±0.0		27.0 ^{ab} ±0.1	
	74.9 ^a ±0.4	74.2±1.8	46.2 ^a ±0.3	47.1±0.8	12.0 ^a ±0.1	11.9±0.3	27.1 ^a ±0.1	26.9±0.2
	72.2 ^a ±0.6		47.4 ^a ±0.1		12.1 ^a ±0.3		26.7 ^b ±0.1	
Post-agricultural Muchocin/3	75.7 ^a ±0.2		46.5 ^a ±0.0		12.0 ^{ab} ±0.3		27.2 ^{ab} ±0.1	
	76.3 ^a ±1.7	75.8±0.3	47.6 ^b ±0.4	47.5±0.7	11.6 ^b ±0.0	11.9±0.3	26.7 ^a ±0.7	27.4±0.7
	76.0 ^a ±0.8		47.9 ^b ±0.8		12.2 ^a ±0.1		28.1 ^b ±0.1	
Forest Goraj/4	77.4 ^a ±0.5		47.3 ^a ±0.3		12.0 ^a ±0.2		26.7 ^a ±0.0	
	74.5 ^b ±1.0	76.6±1.7	46.0 ^{ab} ±0.1	46.5±0.8	12.1 ^a ±0.1	12.2±0.4	27.6 ^b ±0.1	27.1±0.5
	77.4 ^a ±0.8		45.9 ^b ±0.8		12.7 ^a ±0.3		27.0 ^a ±0.1	

Mean values (n = 3) ± standard deviations; identical superscripts (a. b. c..) denote no significant (p < 0.05) difference between mean values in columns according to Tukey's HSD test (ANOVA) for investigated wood samples.

The investigated wood is characterised by a high homogeneity of cellulose content. The range of cellulose content for all the trees varied from 45.9% to 47.9%. The average content for each stand amounted to 47.6% for the trees from Sowia Góra, 47.1% for the trees from Zamyślin and 47.5% and 46.5% for Muchocin and Goraj, respectively. According to Prosiński [1984], the content of cellulose in Scots pine ranges from 41.9% to 54.2%. The obtained results are representative for this species. The high content of cellulose enables the use of the investigated feedstock in pulp and paper technology. Cellulose can be also used in the production of cellophane, cellulose esters e.g. nitrocellulose, cellulose acetate, rayon and cellulose ethers. According to Krzyżaniak et al. [2014], cellulose can be applied as feedstock for the production of highquality cosmetics, pharmaceutical products, tires and others. The levulinic acid

produced during the hydrolysis of carbohydrates is an ingredient of medicines, a plasticizer and a substrate for biofuels. The possibilities of cellulose chemical conversion not only depend on its percentage in wood, but also on its structure. The FTIR investigation indicated that the cellulose from the primeval forest sites had higher TCI and LOI parameters (table 3). The higher parameters showed a higher crystallinity index and order of cellulose structure. As a result, this material was characterised with better mechanical properties, but it underwent chemical conversion with more difficulty.

Table 3. Structural characteristic of cellulose and lignin of *Pinus sylvestris* L. from stands 1-4 by FT-IR

Stand	Cellulose		Lignin	Lignin
	TCI A1372/A2900	LOI A1429/A897	C=O A1710/A1510	Al/Ar A2930/A1510
Primeval forest Sowia Góra/1	1.40	4.13	0.22	0.44
Primeval forest Zamyślin/2	1.37	3.98	0.25	0.44
Post-agricultural Muchocin/3	1.29	3.66	0.22	0.46
Forest Goraj/4	1.32	3.92	0.23	0.45

Other carbohydrate components of a wood tissue also known as arabinoxylans are pentosans. Due to their complex structure, numerous functional groups and the presence of β -d-xylopyranosyl and α -l-arabinofuranose units, pentosans are attractive for chemical synthesis. In the investigated trees the content of pentosans ranged from 11.5% to 12.7% (table 2.) The results for all the trees and the average values are comparable and higher than literature data according to which the content of pentosans in Scots pine wood amounts to 8.2% [Fengel and Wegener 1989]. This increases the possibility of applying this feedstock to the chemical processing of wood in the hydrolysis process for obtaining monosaccharides as xylose or producing biofuels [Bénes et al. 2013]. Hemicelluloses can be applied in the production of ethanol or furfural which can be used as a feedstock to produce non-petroleum derived chemicals, e.g. furfuryl alcohol, methyltetrahydrofuran and furan [Bozell and Petersen 2010; Serrano et al. 2012].

The percentage content of another main wood component: lignin in Scots pine according to literature data is 26.3% to 31.4% [Prosiński 1984]. Lignin is a component whose content changes along with the age of a tree. It influences its stiffness, limits the development of microorganisms and controls water transport in the tree. In the compared trees, the content of this component varied from

26.7% to 28.1% and therefore, it was lower in content values of this component in trees (table 2). This is favourable because during bioethanol or biogas production from biomass it is necessary to conduct pre-treatment processes to partially separate cellulose from lignin and to allow the enzymatic hydrolysis of carbohydrates. Both percentage and structure *i.e.* the content of carbonyl groups as well as the aromatic and aliphatic structures ratio in the investigated lignin were similar in the compared trees (table 3). Lignin holds a great potential in various industries as a source of chemicals, fuels and other bioproducts. Although the calorific value of lignin is similar to ethanol (27 kJ/g), the high density of lignin makes this compound a potential source for bioenergy from high-lignin feedstocks [Vishtal and Kraslawski 2011; Welker et al. 2015].

In the result of pyrolysis of lignin from the comparison sites, the following similar products were obtained: 2-methoxyphenol; 2-methoxy-4-methylphenol; eugenol; trans-eugenol and 2-methoxy-4-(1-propenyl) phenol. However, their total content varied. For pine from Sowia Góra these components were about 70%, from Zamyślin 51%, from Muchocin 39% and Goraj 52% of all the obtained components.

After the pyrolysis of carbohydrates of wood from Sowia Góra the main products were furfural, 3-methyl-1,2-cyclopentanedione, which in total amounted to approx. 13%. As a result of the degradation of carbohydrate components from pine from Goraj the highest peaks came from 2-furanmethanol, 1,2-cyclopentanediol and 3-methyl-1,2-cyclopentanedione. These components were 14%. For carbohydrates from pine from Muchocin the major components were furfural and an unidentified component, in pine from Zamyślin the following: furfural, 2-furanmethanol and 3-methyl-1,2-cyclopentanedione, 22 and 18%, respectively.

The main products obtained after pyrolysis were components from lignin which ranged from 53 to 79% (table 4). The products in all cases were similar but they differed in percentage, which pointed out differences in the chemical structure of the investigated wood. A similar case was stated for products of carbohydrate pyrolysis [Lucejko 2010].

Table 4. Relative percentage of carbohydrates and lignin products of the Py-GC/MS analysis of *Pinus sylvestris* L. from stands 1-4

Compounds	Stand			
	Primeval forest Sowia Góra/1	Primeval forest Zamyślin/2	Post-agricultural Muchocin/3	Forest Goraj/4
Carbohydrates [%]	20.5	37.4	47.3	29.1
Lignin [%]	79.5	62.6	52.7	70.9

Considering the density of the investigated wood [Roszyk et al. 2016] the content of the main components was shown in kg/m³. The obtained results were

presented in figure 2. Such presentation enables the comparison of the chemical composition of a wood volume unit (1 m^3). The highest amount of cellulose in 1 m^3 (238 kg) is found in wood growing in forest stand 1 in Sowia Góra. The same feedstock gives the possibility of gaining the highest amount of lignin (140 kg) and pentosans (62 kg). The second feedstock obtained from forest stand 2 from Zamyślin gives the possibility of obtaining about 10% less of the main components of wood. Figure 3 shows the total content of lignin and cellulose in the investigated wood. On that basis, it was concluded that the trees from Puszcza Notecka vary concerning the content of the main structural components. In the case of the wood gained from the primeval forest stand 1 in m^3 378 kg of lignin and cellulose were found and from the primeval forest stand 2 only 345 kg. The feedstock from the post-agricultural forest stand 3 contains 371 kg/m^3 of the investigated structural components whereas from forest stand area 4, in Goraj – 349 kg/m^3 . Apart from the aspects connected with applying the investigated wood in chemical processing, it has a significant influence on its mechanical properties and as a result, its potential application as solid wood.

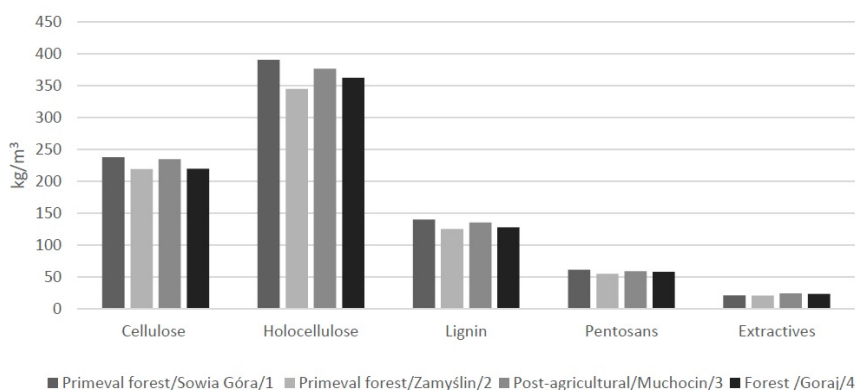


Fig. 2. Average content of components expressed in kg/m^3 of *Pinus sylvestris* L. from stands 1-4 F

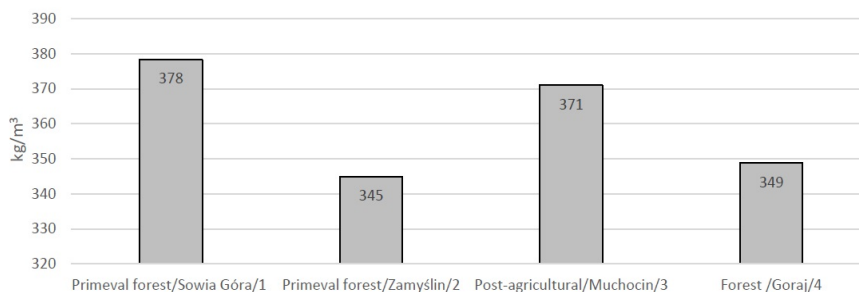


Fig. 3. Total content of cellulose and lignin expressed in kg/m^3 in *Pinus sylvestris* L. from areas 1-4

Conclusions

The quality investigation of Scots pine from Puszcza Notecka, containing the determination of the chemical composition indicated that it is characterised by a lower content of extractives in comparison to trees from post-agricultural and outside of the primeval forest stand. This is a positive feature concerning the production of fibreboards and chipboards. The trees growing in Puszcza Notecka are characterised by a different content of cellulose and lignin. The trees from Sowie Góra which have the highest content of high molecular weight carbohydrates will meet the conditions of the pulp and paper technology better than those growing in Zamyślin. Including the lowest content of lignin and extractives which inhibits the fermentation process, the trees from Zamyślin have better features as a feedstock for the biotechnological processing of biofuels.

The results obtained indicated that the trees from Puszcza Notecka are an attractive feedstock for different technological uses due to homogenous chemical and physical features and they can potentially be used for countless economically-viable applications.

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List of standards

- PN-86/M-04001** Sita tkane kontrolne o oczkach kwadratowych [in Polish]
PN-92/P-50092 Drewno. Analiza chemiczna [in Polish]

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