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AYBEN KILIÇ PEKGÖZLÜ

MUHSİN TAŞ

ESRA CEYLAN

JARL HEMMING

TÜBİTAK

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# Effect of the height of the stem on the polysaccharide composition of *Pinus brutia* (Ten) wood and kraft-pulp

**Ayben KILIÇ PEKGÖZLÜ[1,*], Muhsin TAŞ[1], Esra CEYLAN[1], Jarl HEMMING[2]**

[1]Department of Forest Products Engineering, Faculty of Forestry, Bartın University, Bartın, Turkey

[2]Wood and Paper Chemistry, Åbo Akademi, Åbo, Finland

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**Abstract:** The first goal of this study was to determine the polysaccharide composition of *Pinus brutia* wood and how this composition changes during kraft pulping. The second goal was to determine the effect of the stem height on chemical composition. The content of hemicelluloses was about 280 mg/g at a height of 1.3 m, whereas the content was about 300 mg/g at the top of the stem (4.3 m). The main sugars in the wood of *P. brutia* were mannose (102 mg/g dw), xylose (69 mg/g dw), and glucose (44 mg/g dw). During kraft pulping, 52% of the total hemicelluloses were degraded. Glucose and xylose were more stable than mannose. The total amounts of cellulose in the wood and in the kraft pulp of *P. brutia* were determined to be 330 mg/g and 661 mg/g, respectively, at 1.3 m.

**Key words:** Hemicelluloses, kraft pulp, stem height, Turkish pine

## 1. Introduction

The increasing scarcity of resources and the increasing global population are pushing mankind to find new renewable resources. Cellulose, hemicelluloses (HCs), and lignin are the most abundant biopolymers found in nature. Cellulose is a linear homopolysaccharide composed of glucose units. The amount of cellulose in wood is 40%–50%. HCs are heteropolysaccharides that are composed of pentoses, hexoses, and uronic acids in the pyranose and furanose forms. Depending on the sources of the biomass, the levels of HCs are 15%–35%. HCs have branched and amorphous structures. In woody plants, HCs are found in the primary and secondary cell walls (Fengel and Wegener, 2003; Teleman et al., 2009; Kapu and Trajano, 2014). The most abundant HCs are xylans and glucomannans. Xylan is the master unit in hardwoods (25%–35% dry mass) and herbaceous plants. However, mannan is the dominant unit occurring in the form of glucomannans and galactoglucomannans (GGMs) in softwoods (Mikonen and Tenkanen, 2012). GGMs consist of β–D-glucopyranosyl and β–D-mannopyranosyl units that are linked linearly with α–D-galactopyranose units attached to the linear chain by α-(1,6) bonds. The percentage of mannan in softwood is 10%; galactan is 3% of the dry mass of the raw material. Other hemicelluloses are arabinoglucoronoxylans (AGXs), found in smaller amounts in softwoods (5%–10% of dry mass). The linear backbones of AGXs consist of β-(1,4) –D-xylopyranose units with α–L-arabinofuranose and 4-O-metil–α-D-glucopyranosyl uronic acid linkages (Girio et al., 2010; Kapu and Trajano, 2014; Persson and Jönsson, 2017).

Compared to cellulose and starch, HCs have limited usage in industry, even though they are abundant. Generally, they are used as an energy resource with lignin, although HCs have only about half of the heating value of lignin i.e. the heating values for lignin and HCs are 27 MJ/kg and 13.6 MJ/kg, respectively (Yoon and van Heiningen, 2008). HCs that are recovered before the pulping process can be used in various industrial applications. Biorefineries can take a step toward the utilization of HCs in different areas (Vila et al., 2011). HCs can be used as an alternative to petroleum-based polymers. The features of film-forming and biodegradability make xylan and mannan a resource for food packaging. They have low oxygen, grease, and permeability to various aromas, as well as high tensile strength. HCs are used for coating fruit, cheese, and paper. In addition to being edible biosurfactants and food additives, HCs are utilized in hydrogels, contact lenses, and the coatings for controlled-release drugs (Karaaslan et al., 2011; Li et al., 2013; Li et al., 2017). Because of their hydrophilic structures, which have positive effects on their strength properties, they can also be used as additives in the production of paper (Bai et al., 2012; Liu et al., 2015). The extraction of HCs before processing to prevent the

* Correspondence: akilic@bartin.edu.tr

enzymatic hydrolysis of cellulose is important in the production of bioethanol and other bioproducts. HCs are extracted by treating the biomass with acid, water, steam, or mild alkaline solutions. In recent years, commercial production of these bioproducts has begun with the extraction of the HCs (Girio et al., 2010; Jun et al., 2012).

*Pinus brutia*, which is known as Turkish Pine, is a native pine species over an area that exceeds 5,610,215 ha in Turkey. These trees reach heights of 20–25 m, have diameters of up to 60 cm, and have clear annual rings and heartwood (Berkel, 1970; Merev, 2003). The wood of *Pinus brutia* is used as a construction material for poles and fences. It is also suitable for pulp and paper production due to its high yield of fibers. Tannin and resin obtained from the trees are also an important source of income (Göksel, 1981; Öktem, 1987; Tank et al., 1990; Öktem and Sözen, 1995). The chemical composition and anatomical structure of trees significantly affect their usage e.g., in particleboard or pulp and paper industries (Baharoglu et al., 2013; Carillo et al., 2018). These features can vary between different species of trees as well as within a single species (Sjöström, 1993).

In the last few years, the extraction of HCs in biorefineries and prior to pulping processes has become more important due to their increasing usage in various areas and decreasing adverse effects on the quality of bioethanol, as mentioned above. The goal of this study was to characterize the polysaccharide composition and the effect of the height of the trees on the chemical structure and composition.

## 2. Materials and methods

### 2.1. Materials

Samples were taken from Topluca, Bartin Province in Turkey in 2016. Three healthy trees were cut, and each stem was divided into 3 sections (A: 1.3 m; B: 2.8 m; C: 4.3 m). Each section was 50 cm in length. They were debarked and subdivided into 3-cm disks.

### 2.2. Method

#### 2.2.1. Pulping conditions

The kraft (sulphate) method was used as a pulping method. The air-dried chips were cooked in a laboratory-type 15-L electrically-heated rotary digester under the following conditions: 18% active alkali, 25% sulfidity; liquor/wood ratio of 4/1, 170 °C cooking temperature; time for reaching maximum temperature was 90 min; cooking time was 75 min. To remove the black liquor, pulp was washed and disintegrated before screening with a Somerville-type pulp screener with a 0.15-mm slotted plate (TAPPI, 2002b). The pulp samples were stored in a freezer until they were analyzed.

#### 2.2.2. Chemical analysis

For the chemical analysis, the disks were splintered, freeze-dried, and then ground in a Wiley mill. The ground wood was sieved, and 60 mesh samples were used. Table 1 lists the standard methods used in the chemical analysis. For the solvent solubility, ethanol was used, and extraction was performed in a Soxhlet apparatus for 6 h.

Acid methanolysis was used to determine the hemicelluloses (Sundberg et al., 1996).

Both 10 mg of freeze-dried wood flour and pulp fiber were separately weighed and treated with 2 mL of 2M HCL in anhydrous methanol in an oven at 100 °C for 5 h. After samples were cooled to room temperature, 4 mL of resorcinol and 100 µL of pyridine were added. From this aliquot, 1 mL was taken and evaporated under nitrogen before the silylation. For the silylation, 100 µL of pyridine, 150 µL of HMDS (hexamethyldisilazane), and 80 µL of TMCS (trimethylchlorosilane) were used. Resorcinol was used as an internal standard. For the determination of the calibration factor of methanolysis, calibration mixture containing equal amounts of 0.2 g of D-arabinose, L-rhamnose, D-xylose, D-galactose, D-glucose, D-mannose, D-glucuronic acid, D-galacturonic acid was analyzed with acid methanolysis as mentioned above. Analyses were done parallel to each sample. Calibration factor was calculated by dividing peak area of individual sugar units into area of resorcinol peak.

Cellulose is stable to methanolysis (Sundberg et al., 1996); therefore, acid hydrolysis was performed to determine the glucose units coming from the cellulose. Ten mg of wood flour and pulp fiber were treated separately with 0.2 mL of 72% $H_2SO_4$ for 2 h; 0.5 mL of distilled water was added, and it was left for 4 h. At the end of the 4 h, 6 mL of distilled water was added, and the solution was left overnight. On the second day, samples were autoclaved at 125 °C for 90 min. After cooling to room temperature, 1–2 drops of bromine creosol were added as an indicator. For the neutralization, $BaCO_3$ was used. After the color changed to blue, 1 mL of sorbitol was added as an internal standard. A 1-mL aliquot was taken from the sample; 1 mL of acetone was added to this in a new test tube before evaporation under the nitrogen. Samples were silylated as mentioned above. For the determination of calibration factor of acid hydrolysis, 10 mg of cellulose cotton linter was analyzed parallel to wood and pulp samples (Sundberg et al., 2003). For the calculation, the total area of glucose units was divided by area of standard (sorbitol) and then multiplied by the concentration of standard and total amount of sample.

Both acid methanolysis and acid hydrolysis samples were analyzed with FID-GC after the silylation. The amount of glucose was calculated by subtracting the glucose units that came from the hemicellulose.

**Table 1**. Standard methods used in chemical analysis.

| Experiments | Standards |
|---|---|
| Sample preparation | TAPPI T 257 |
| Holocelluloses | Wise and John, 1952 |
| α-Celluloses | Rowell, 2005 |
| Lignin | TAPPI T 222 |
| Cold–hot water solubility | TAPPI T 207 |
| Ash content | TAPPI T 211 |

**2.2.3. Chromatographic conditions**

A gas chromatograph (Shimadzu GC-2010) coupled with an HP-1 (J & W) 25 m × 0.20 mm (ID) × 0.11 μm film thickness column was used with a flame ionization detector. The carrier gas was $H_2$. The temperature program for methanolysis was 100 °C for 8 min, then raised to 170 °C at the rate of 2 °C/min, followed by increasing the temperature at the rate of 12 °C/min to 290 °C (15 min). The temperature of injector and detector were 250 °C and 300 °C, respectively. The acid hydrolysis began at 100 °C and the temperature was raised to 175 °C at the rate of 4 °C/min. Subsequently, the temperature of the acid hydrolysis was raised from 175 °C to 290 °C at the rate of 12 °C/min rate.

**2.2.4. Statistical analysis**

The SPSS statistics software package was used for the analyses. The collection of statistic models known as analysis of variance (ANOVA) was used to test the effect of the height of the stem on the chemical composition of the wood at the 5% significance level. Duncan's multiple range tests were used.

## 3. Results and discussion

Table 2 gives the mean values of the components of the main cell wall of Turkish pine (i.e. holocelluloses and lignin) and the mean values of the low-molecular compounds (extractives and ash). It is evident that the results are compatible with the values reported in the literature (Hafizoglu and Usta, 2005; Kilic et al., 2010). Some values, e.g., solubility and the amount of cellulose, could vary because of the differences in the location and age of the tree.

The height of the stem affects the main cell wall components statistically (Figure 1). The amount of α-cellulose decreased as the height of the stem increased. The percentages of α-cellulose at breast height (1.3 m), in the middle (2.8 m), and at the top of the stem (4.3 m) were 47%, 46.5%, and 45.8%, respectively. The cellulose content at the top of the tree (C) was significantly different from the contents at A and B. Similar results were detected in black pine; i.e. the α-cellulose at breast height and at the top of the stem was 52% and 49.7%, respectively (Pekgözlü Kilic et al., 2017). Contrary to the pattern of cellulose content, the lignin contents were only 25.6% and 26.6% at the bottom (A) and top (C) of the stem, respectively. The lignin content at the top was statistically different from the lignin contents at A and B. Kostiainen et al. (2004) reported similar results between lignin and the 3 stem heights for Norway spruce i.e. lignin contents at breast height and at the top were 28.4% and 30.2%, respectively. It is well known that juvenile wood has high lignin and hemicellulose contents and low amounts of cellulose (Camphell et al., 1990; Caron et al., 2013; Krutul et al., 2014a; Salazar et al., 2015). High α-cellulose content is an important feature for enhancing the yield during pulping, and the lignin content affects the cooking parameters and the amounts of chemicals.

**Table 2**. The chemical composition of *P. brutia* wood (%); $P < 0.05$.

| Experiments | Mean | Kilic et al. (2010) | Hafizoglu and Usta (2005) |
|---|---|---|---|
| Holocelluloses | 73 ± 1.1 | 72 | - |
| α-Cellulose | 46 ± 0.2 | 46 | 50 |
| Lignin | 26 ± 0.3 | 27 | 29 |
| Solvent solubility | 2.2* ± 0.1 | 2.3* | 5.7+ |
| Hot water solubility | 4.4 ± 0.2 | 3.2 | 5.1 |
| Cold water solubility | 3.5 ± 0.1 | 2.3 | 2.4 |
| Ash | 0.1 ± 0.01 | 0.3 | - |

*: alcohol ; +: alcohol–benzene.

No linear correlation was observed between the solubility and the ash content of Turkish pine (Figure 2). Neverova et al. (2013) reported that the extractives were higher in the bottom part of the larch stem. However, in the European larch, the solubility and the amount of hemicelluloses decreased and the ash content increased as the height on the stem increased (Muhcu et al., 2015). However, in oak wood, the extractive content increased as the height of the stem increased (Krutul et al., 2014b).

Table 3 shows that the total amount of sugar units that form the composition of hemicelluloses in the wood of Turkish pine trees was between 280 mg/g and 300 mg/ g. There was a slight increase in the total amount of hemicelluloses with the height of the stem. The mean value of hemicelluloses in Turkish pine wood was about 290 mg/g. This value was 284 mg/g in *P. sylvestris* wood and 290 mg/g in *P. resinosa* wood (Cote, 1966; Willför et al., 2005). In the cones of the same *Pinus* species, the amount of total hemicelluloses was about 250 mg/g (Kilic et al., 2010).

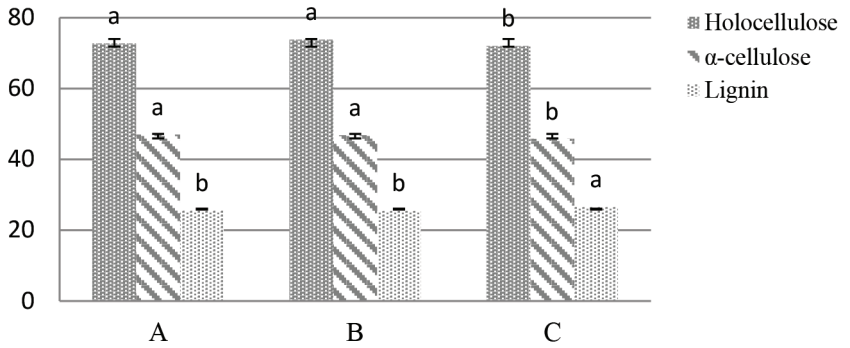


**Figure 1.** Effect of the height of the stem on the chemical composition of *P. brutia* wood (%).

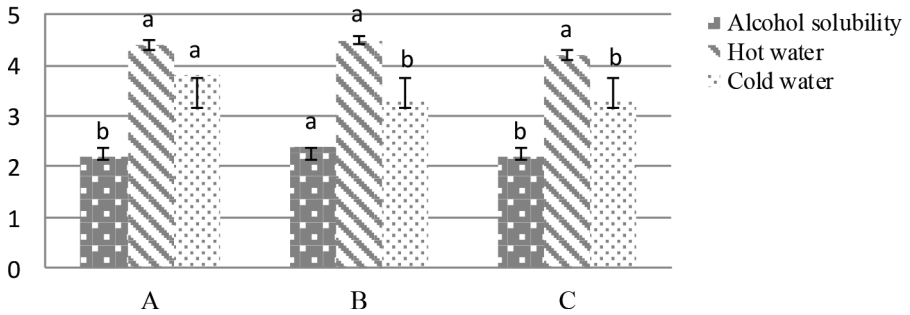


**Figure 2.** Effect of the height of the stem on the solubility values of *P. brutia* wood (%).

**Table 3**. Amount of sugar units in the wood and pulp of *P. brutia* (mg/g dry sample).

| Samples | | Ara | Xyl | Gal | Glc | Man | Rha | GalA | GlcA | 4-O-MeGlcA | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Wood | A | 15 ± 0.7 | 66 ± 0.8 | 26 ± 1.0 | 44 ± 0.4 | 103 ± 2.7 | 3.0 ± 0.1 | 16 ± 1.1 | 4.8 ± 0.2 | 2.4 ± 0.1 | $280^{a}$ ± 4.4 |
| | B | 16 ± 0.8 | 68 ± 0.5 | 26 ± 0.4 | 43 ± 0.4 | 102 ± 1.7 | 3.1 ± 0.2 | 16 ± 0.7 | 5.0 ± 0.3 | 2.7 ± 0.1 | $282^{a}$ ± 1.9 |
| | C | 17 ± 0.3 | 74 ± 0.5 | 28 ± 0.7 | 44 ± 0.4 | 102 ± 2.6 | 3.5 ± 0.2 | 22.7 ± 0.6 | 5.7 ± 0.0 | 3.1 ± 0.2 | $300^{b}$ ± 3.6 |
| Pulp | A | 5.7 ± 0.4 | 49 ± 1.9 | 5.6 ± 0.5 | 45 ± 2.4 | 30 ± 1.1 | 1.1 ± 0.2 | 0.0 | 0.0 | 0.0 | $137^{ab}$ ± 5.5 |
| | B | 6.6 ± 0.1 | 54 ± 0.1 | 5.7 ± 0.1 | 44 ± 0.4 | 31 ± 0.6 | 1.2 ± 0.1 | 0.0 | 0.0 | 0.0 | $142^{b}$ ± 1.1 |
| | C | 6.0 ± 0.4 | 52 ± 1.3 | 5.2 ± 0.3 | 41 ± 1.4 | 27 ± 1.3 | 1.1 ± 0.3 | 0.0 | 0.0 | 0.0 | $132^{a}$ ± 4.3 |

Mannose was found to be the major sugar unit, making up almost 35% (w/w) of total hemicelluloses, while xylose, with 24% (w/w), was the second most important unit. The combined amounts of the acidic group, i.e. galacturonic acid, glucuronic acid, and 4–O-methylglucuronic acid, were about 23 mg/g and 32 mg/g. Except for mannose and glucose, the amounts of all other sugar units increased as the height of the stem increased, especially between A and C. There was more xylose in the juvenile wood but there was more mannose in the mature wood (Johansson et al., 2015).

In the softwood, galactoglucomannan (GGM) is composed of galactose, glucose, and mannose, which are the predominant hemicelluloses. According to the species of the tree and its growth stage, some structural differences can be found.

In Turkish pine wood, the ratio of sugars was Gal (1):Glc (1.7):Man (4). This ratio is referred to as a galactose-rich water-soluble GGM polymer (Willför et al., 2003; Ebringerova et al., 2005; Mikonen and Tenkanen, 2012). Pectin is another polysaccharide found in Turkish pine wood and it is composed mainly of galacturonic acid and rhamnose units (GalA: Rh = 5–6:1). Pectin gives an anionic charge to fibers, which is beneficial in the paper industry, especially with respect to the strength of paper (Hamaguchi et al., 2013).

A low degree of polymerization and the amorphous structure of hemicellulose cause degradation during kraft

pulping. In this study, 52% of the total hemicelluloses in Turkish pine was degraded. In softwoods, the degradation begins as soon as the cooking begins, due to the reduction of the reducing end groups of galactoglucomannans. More than 71.4% of the mannose was lost. However, the decrease in xylose content was only 17.5% because of its strong hydrogen bonds with cellulose and other hemicelluloses, like 4–O-MeGlcA (Hamaguchi et al., 2013; Johansson et al., 2015). The acidic groups (e.g., GalA, GlcA, and 4-O-MeGlcA) were completely destroyed. As mentioned above, they are pectin substances, and they dissolve easily in an alkaline medium (Figure 3). The glucose that came only from the hemicelluloses was the only sugar unit that was not affected by kraft cooking. With regard to the height of the stem, no clear statistical difference was observed in the total amount of hemicelluloses in the pulp.

The total amount of cellulose determined by acid hydrolysis was 330 mg/g in the wood and 661 mg/g in the kraft pulp of *P. brutia* at breast height (Figure 4). No correlation was observed in the wood between the cellulose content and the height of the stem. The highest cellulose content was observed at the height of 2.8 m (B). In the gravimetric analysis, A and B were similar statistically, but C was different in that it had the lowest amount. It could be that during the gravimetric analysis, not only α-cellulose but also some β- and δ-cellulose were dissolved. However, in the kraft pulp at the height of 2.8 m (B), the cellulose content decreased about 646 mg/g dw compared to the content at breast height.

## 4. Conclusion

The increasing interest in natural renewable resources has made celluloses and hemicelluloses more important. The aim of this study was to determine the composition of hemicelluloses in *Pinus brutia* wood and how their composition is affected by the height of the stem and during kraft pulping.

The total mean value of hemicelluloses in *Pinus brutia* wood was 287 mg/g dry sample. The most abundant noncellulosic sugar units were mannose (102 mg/g) and xylose (69.5 mg/g). From the acidic carbohydrates, galacturonic acid (18.3 mg/g dry sample) is the most abundant unit, followed by glucuronic acid (5.2 mg/g dry sample). With the stem height, a slight increase was

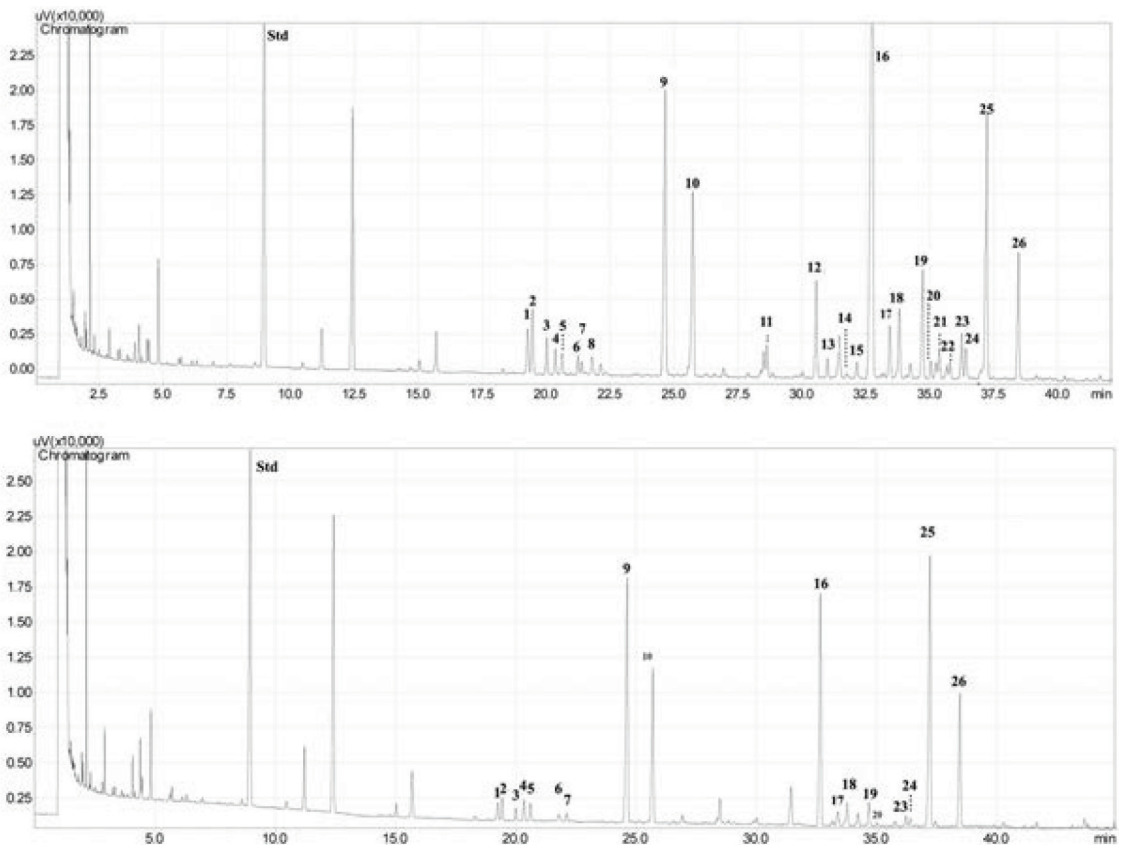


**Figure 3.** Acid methanolysis chromatograms of wood and kraft pulp of *P. brutia* Arabinose: 1, 2, 3, 8; Xylose: 4, 5, 9, 10; Rhamnose: 6, 7; Mannose: 16, 18, 24; Galactose: 17, 19, 20, 23; Glucose: 25, 26; Galacturonic acid: 12, 15, 21, 22; Glucuronic acid: 11, 14; 4–O–Methyl-Glucuronic acid; Std: Resorcinol.

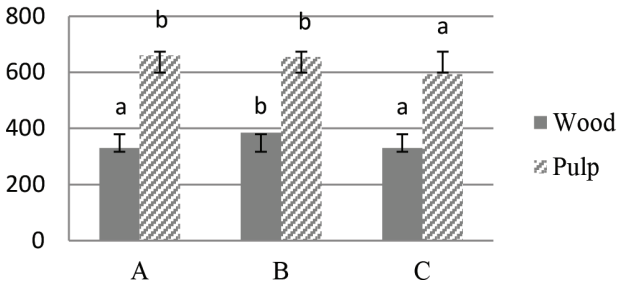


**Figure 4.** Cellulose content of *P. brutia* wood and pulp (mg/g dry sample).

observed in the total amount of hemicelluloses. At the top of the tree, the total amount of hemicelluloses was high.

Total mean value of cellulose in *Pinus brutia* wood was 330 mg/g dry sample. In the gravimetric analysis, α-cellulose decreased with stem height, and lignin content increased.

During kraft pulping, all pectic substances and 70% of mannose were degraded. Degraded sugar units potentially can be used in the food and pharmaceutical industries. This can be considered for biorefineries, and may increase sustainability of forestry.

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