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Seasonal dynamics of polysaccharides in Norway spruce (*Picea abies*)

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ABSTRACT

Annual dynamics of accumulation and changes in the monosaccharide composition of pectin-, arabinan- and galactan-containing polysaccharides and binding glycans isolated from greenery (thin branches with needles) of Norway spruce were investigated in this study. The polysaccharides were compared with polysaccharides of Siberian fir according to the yields, composition and content of typical components. It was shown that Norway spruce greenery contains lowly methyl-esterified pectin extracted with ammonium oxalate, which is a part of protopectic complex and is bound with components of cell walls via ionic bonds. In contrast, Siberian fir greenery contains mainly water-extracted highly methyl-esterified pectin, weakly bound to cell wall components. It was concluded that an autumn-winter period is the optimal time for harvesting Norway spruce and Siberian fir greenery for isolation of the pectic polysaccharides. The revealed regularities indicate that there is a certain biorhythm of accumulation of the compounds, probably determined by genetic factors.

1. Introduction

Forest resources in Russia, mostly formed by conifer forests, are the principal factor shaping the Russian nature and providing various useful products. The analysis of the literature on the structural chemistry of polysaccharides has shown that the polysaccharides of conifers (except cellulose and binding glycans) are the least studied polysaccharides, in spite of the demand and economic importance of this raw material (Makarova, Shakhmatov, Udoratina, & Kutchin, 2015).

Phloem and cambium, sapwood and heartwood, tissues of a tree trunk, are the principal objects of studies of the polysaccharide composition of wood of conifers. The phloem and cambium are characterized by a high content of pectin (4.4–18%), while in the sapwood and heartwood its content is low (0.5–3.8%) (Makarova et al., 2015; Thornber & Northcote, 1961a, 1961b; Willför, Sundberg, Hemming, & Holmbom, 2005).

The monosaccharide composition and content of water-soluble polysaccharides in the sapwood and heartwood have been studied for the most widespread types of coniferous trees belonging to the genus Abies of the Pine family (*Abies balsamea*, *Abies sibirica, Abies lasiocarpa*), larches (*Larix lariciana, L. sibirica*, *Larix decidua, L. occidentalis*), pines (*Pinus banksiana, Pinus resinosa, Pinus silvestris, Pinus taeda, Pseudotsuga menziesii*), spruces (*Picea abies, Picea glauca, P. rubens*, *Picea mariana*), and the genus Thuja in the cypress family (*Thuja occidentalis*). The greatest amount of pectic polysaccharides (1.5%) was isolated from wood of *A. balsamea* (Bertaud & Holmbom, 2004; Goellner et al., 2011; Karácsonyi, Kováčik, Alföldi, & Kubačková, 1984; Odonmazig, Ebringerová, Machová, & Alföldi, 1994; Ponder, 1998; Willfor & Holmbom, 2004; Willför, Sjöholm, Laine, & Holmbom, 2002; Willfor et al., 2005).

Complex utilization of forest resources involves the use of whole biomass of trees as well as utilization of wood waste generated during harvesting and wood processing at logging enterprises. Pectic polysaccharides were found in bark of various species of coniferous trees, e.g., *Abies amabilis* (Bhattacharjee & Timell, 1965), *Pinus pinaster* (Fradinho et al., 2002), *P. sylvestris* (Valentín et al., 2010), *L. sibirica, Larix gmelinii* (Trofimova, Medvedeva, Ivanova, Babkin, & Malkov, 2012), and *P. abies* (Bianchi et al., 2015; Kemppainen, Siika-Aho, Pattathil, Giovando, & Kruus, 2014; Krogell, Holmbom, Pranovich, Hemming, & Willför, 2012; Le Normand et al., 2014). Meanwhile, it is known that biological processes actively take place in storage organs of coniferous trees, particularly in needles, where metabolites, including pectins, are accumulated and spent during perennial cycles for growing of the vegetative mass (Robakidze & Bobkova, 2003). In the total amount of waste generated by different branches of wood-processing industry, the proportion of wood greenery is 20–30 million tons per year (Yagodyn, 2001). Coniferous greenery is a perspective raw material resource for production of biologically active substances for therapeutic and prophylactic applications due to the possibility of year-round using and sufficient availability of the raw material base.

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It is known that wood greenery contains much more pectic compounds than wood of a trunk (Shakhmatov, Udoratina, Atukmaev, & Makarova, 2015; Willfor et al., 2005). However, the data on the composition and structure of the polysaccharides of storage organs of conifers is limited up to the present time, despite the potential economic importance of this raw material (Makarova et al., 2015; Shakhmatov et al., 2015).

As a result of our previous studies, it was found that greenery of Siberian fir *A. sibirica* is a potential source of pectin (content of up to 8%). An effective method of obtaining pectin polysaccharides and binding glycans from coniferous greenery was developed and patented on the example of *A. sibirica* (Patova, Makarova, & Shakhmatov, 2011). The previous studies encompass the annual dynamics of accumulation and the character of change of the monosaccharide composition of polysaccharides of greenery of *A. sibirica*. It was shown that an autumn period is optimal for harvesting coniferous greenery to isolate pectic polysaccharides (taken into account the content of uronic acids, protein and starch), and a winter-spring period is optimal to isolate the binding glycans (Makarova, Patova, Mikhailova, & Demin, 2011; Shakhmatov et al., 2015).

Meanwhile, it is known that the monosaccharide composition and structural organization of the polymers of plant cell walls may vary not only between different species of plants, but also between different tissues of a plant. In this regard, the study of polysaccharides of Norway spruce (*P. abies*), a widely distributed forest species, is of great interest. Comparison of the results with those previously obtained for fir might reveal similarities and differences of these sources of biologically active substances (e.g. polysaccharides).

The Norway spruce (*P. abies*) is a large evergreen coniferous tree of the genus *Picea* in the family *Pinaceae*. Wood greenery of *P. abies*, large-tonnage waste of wood-processing industry, is relatively new, insufficiently studied, non-traditional raw material to obtain pectic polysaccharides. Efficient use of spruce greenery is impeded by the lack of knowledge about the dynamics of accumulation, composition and structure of the cell wall polysaccharides. Meanwhile, a purposeful study of structural features of components of *P. abies* greenery and their chemical characteristics can help to develop the scientific basis of the greenery processing, which is crucial for determining prospects of application of the greenery.

This study presents the analysis of the dynamics of accumulation and the character of change of monosaccharide composition of polysaccharides of *P. abies* greenery during a year. The yields, composition and content of typical components (uronic acid, neutral sugars) of the spruce greenery polysaccharides were compared with respective characteristics of previously studied polysaccharides of greenery of Siberian fir *A. sibirica*.

2. Materials and methods

2.1. Preparation of plant raw material and isolation of polysaccharides

Coniferous greenery of *Picea abies* was collected near Syktyvkar (Komi Republic, Russia) in the period from January to December 2013 (except August). The samples were taken from 10 to 20 growing trees in the middle of each month. Such biological replication gives a representative sample providing the 5% level of significance (Sudachkova & Semenova, 1971). For analysis of coniferous greenery, thin branches (less than 8 mm in diameter) with needles, from the top, middle and bottom sections of a tree crown were cut in four different geodesic directions.

The isolation was performed according to the procedure described earlier (Shakhmatov et al., 2015). The residual raw material (50 g) was extracted five times with distilled water (1 l) under continuous stirring for 2 h at 70 °C (Fig. 1). The combined extract was filtered, concentrated and centrifuged. The supernatant was collected and precipitated with four volumes of 96% ethanol. The precipitate was separated by centrifugation and redissolved in water. The resulting polysaccharide PA_W was dialyzed and freeze-dried.

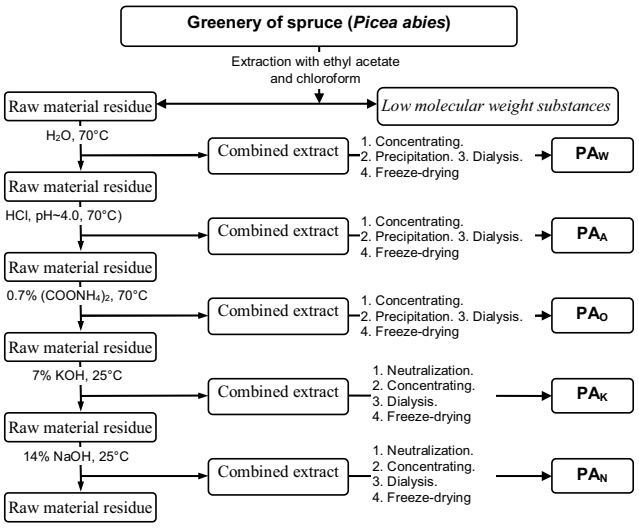

Fig. 1. Scheme of extraction of greenery of Norway spruce.

Hydroalcoholic supernatants obtained by ethanol precipitation of PA_W fractions were combined, concentrated and dialyzed against distilled water (3.5 kDa membrane). The resulting solution was centrifuged, concentrated and freeze-dried. As a result, polysaccharide fractions PA_W-S were obtained (corresponding to the June–December period).

The residual material was treated with dilute hydrochloric acid at pH ~3.5–4 (1 l) with continuous stirring and heating to 70 °C for 2 h five times. The combined extract was treated as described above. As a result, the polysaccharide PA_A was obtained.

Hydroalcoholic supernatants obtained by ethanol precipitation of PA_A fractions were combined, concentrated and dialyzed against distilled water (3.5 kDa membrane). The resulting solution was centrifuged, concentrated and freeze-dried. As a result, polysaccharide fractions PA_A-S were obtained (corresponding to the June – December period).

The residue was treated with 0.7% aqueous solution of $(NH_4)_2C_2O_4$ (1 l) with continuous stirring at 70 °C for 2 h five times. The combined extract was treated as described above. As a result, the polysaccharide PA_O was obtained.

The residual material was extracted five times with 7.0% aqueous solution of KOH (with 10 mmol/L $NaBH_4$; 0.5 l) with stirring at 25 °C for 2 h. The combined extract was cooled, acidified with acetic acid to pH 5.0 and centrifuged. The purified extract was concentrated, dialyzed, centrifuged and freeze-dried. After that, the polysaccharide PA_K was obtained.

The residue was extracted five times with 14% aqueous solution of NaOH (0.5 l) contained 10 mmol/L $NaBH_4$ and 4% H_3BO_3 with stirring at 25 °C for 2 h. The combined extract was treated as described above for the polysaccharide PA_K. As a result, the polysaccharide PA_N was obtained.

2.2. General analytical methods

The glycuronic acid content was determined by reaction with 3,5-dimethylphenol in the presence of concentrated sulphuric acid. A calibration plot was constructed for D-galacturonic acid