HOT-WATER EXTRACTION AND CHARACTERIZATION OF HEMICELLULOSES AND PECTINS FROM BARK OF NORWAY SPRUCE (PICEA ABIES)

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ABSTRACT
Bark of Norway spruce (Picea abies) contains 20 to 30% of hemicelluloses and pectins which could be extracted with pressurized hot water. Hemicelluloses and pectins from the fresh inner bark were extracted with an Accelerated Solvent Extractor (ASE) with water at 100 °C, 140 °C and 160 °C. A large amount of arabinose and galacturonic acid units in the water extract obtained at 140 °C revealed the presence of arabinans and pectins. At this temperature, the extraction of hemicelluloses and pectins was the most effective and generated high-molar-mass non-cellulosic polysaccharides with an average molar mass $M_w$ around 40 kDa. Aromatic substances present in the hot-water extract at 140 °C could partly be removed by sorption on DAX-8 polyacrylate resin.

I. INTRODUCTION
Bark is a side stream of the pulp and paper industry and is habitually used as a fuel. Heat coming from the combustion of bark is partially used for production of thermal and/or electrical energy on-site. The quantities of bark available are considerable. For instance, the industrial processing of softwood in Sweden gives about 2 Mtons/year of pine bark and 4 Mtons/year of spruce bark. (Swedish Forest Agency, report 2008). Our idea is to extract bark prior to its combustion and exploit and upgrade the extracts to different value-added chemicals.

Apart from energy production, softwood barks have a variety of markets as soil conditioners and amendments, and as decorative landscape mulching products. Bark tannins are also used as an adhesive and natural binder for medium density fiberboards (MDF) (Xing et al. 2007).

Bark is a highly complex and heterogeneous material. It consists of the inner bark and the outer bark, which have different functions and chemical compositions and should therefore be studied separately. However, since the separation of inner and outer bark is tedious, and is difficult in industrial practice, investigations have mostly been made on the whole bark. Back and Lundqvist (1975) showed that spruce bark contained 35 % lignin, 35 % cellulose and 20 % hemicelluloses.

There are numerous reports on hemicelluloses in wood. In Norway spruce wood, the predominant hemicelluloses are acetyl-galactoglucomannans and -arabinoglucuronoxylans. The structure of hemicelluloses and pectins in spruce bark, however, is not well-characterized. Pectins are heteropolysaccharides with galacturonan and rhamnogalacturonan backbones and also contain in many case arabinose units.

There are numerous reports on the biomedical effects of fruit and plant pectins such as antidiarretic, immunostimulating, antiulcer and antimetastasis actions (Yamada 1996). Pectins are also widely used in the food industry as thickening agents, stabilizers and emulsifiers in jams and sweets (Rolin 2002).

II. EXPERIMENTAL
Bark material
Bark of Norway spruce was sampled from a fresh 30-year-old tree cut in Gävleborg County (Sweden) in July 2009. Inner and outer bark was manually separated using a scalpel.

Extraction
The inner bark was ground with a hand blender to a particle size of approximately 5×2 mm and smaller. The ground bark was sequentially extracted using an Accelerated Solvent Extractor (ASE) (Dionex, California) with acetone followed by hot water at 100 °C, 140 °C and 160 °C. Totally 90 g of wet bark was extracted with ASE with a load of approximately 10 g per cell. The acquired extract volumes were measured and then frozen for transportation and storage.
Carbohydrate analysis of extracts
Total amounts of carbohydrates in the extracts were determined with acid methanolysis and gas chromatography (GC) as described by Sundberg et al. (1996) and Willför et al. (2009). Approximately 0.3 ml of sample was dried and 2 ml of 2 M HCl in methanol was used for the methanolysis (105 °C, 3 h). Pyridine was used to neutralize the samples after the methanolysis. Sorbitol in ethanol (0.1 mg/ml) was used as internal standard. After evaporation of methanol and pyridine, the samples were silylated with HMDS and TMCS. The samples were left to sediment for 16 h and the liquid phase was then transformed into vials for GC analysis.

Fractionation
A 50 ml aliquot of the 140 °C hot-water fraction was eluted through a 50×3 cm column packed with a Supelite™ DAX-8 macroporous polycrylate resin in order to remove aromatic compounds. Polysaccharides were eluted with water until the phenol-sulfuric acid test gave a negative response. The aromatic compounds retained by the resin were then eluted using methanol. Removal of aromatics was assessed by UV spectroscopy at 280 nm.

Klason-lignin
Klason-lignin analyses were performed using sulfuric acid hydrolysis at 125°C for 1 hour and determined gravimetrically.

SEC analysis
Alkaline SEC was used to evaluate the molecular-mass average and the polydispersity of the polysaccharides obtained by hot-water extractions. The SEC system consisted of three TSK gel columns (Tosoh Bioscience, Tokyo, Japan) coupled in series (G3000PW, 7.5× 300 mm, 10 µm particle size; GP4000PW, 7.5 × 300 mm, 17 µm particle size; G3000PW) and a refractive index detector (Waters 2410). The mobile phase was 10 mM NaOH with a flow of 1 ml/min. The system was calibrated using polyethylene glycol and polyethylene oxide standards with molecular masses ranging from 200 to 50 000.

III. RESULTS AND DISCUSSION
Extraction yields
The results of the extraction yields are summarized in Table 1. Almost 70 % (dry weigh) of the fresh inner bark of Norway spruce was dissolved with acetone and hot water at 100-160 °C. Extraction with acetone removed the extractives (mainly stilbene glycosides) and part of the tannins (results not shown). Most of the hot-water-soluble carbohydrates released from fresh bark were oligo- and polysaccharides and only 1 to 2% (w/w dry bark) was present as free monosaccharides. The highest extraction yield was reached at 140 °C where over half of the hemicelluloses and pectins could be extracted. The last extraction step, with hot water at 160 °C gave a low yield compared to the previous steps, indicating that only little hemicelluloses and pectins are left in the bark after extraction with hot water at 140 °C.

<table>
<thead>
<tr>
<th>Extraction</th>
<th>% of dry bark</th>
<th>Hemicelluloses and pectins % of dry bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>15.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Water 100 °C</td>
<td>19.9</td>
<td>8.3</td>
</tr>
<tr>
<td>Water 140 °C</td>
<td>26.7</td>
<td>20.3</td>
</tr>
<tr>
<td>Water 160 °C</td>
<td>7.9</td>
<td>4.2</td>
</tr>
<tr>
<td>Total</td>
<td>70.3</td>
<td>35.3</td>
</tr>
</tbody>
</table>

Carbohydrate composition
Methanolysis on the starting bark material showed that fresh spruce bark contained 43 % of non-cellulosic carbohydrates. After extraction with acetone and hot water, the residue contained only 5 % of carbohydrates, other than cellulose. Thus, almost 90% of the non-cellulosic bark carbohydrates had been successfully extracted. The carbohydrate compositions of bark hemicelluloses and pectins for each extraction step are illustrated in Figure 2. These results showed once more the high extraction yield obtained with hot water at 140 °C. The extracted polysaccharides were mainly composed of glucose, galacturonic acid and arabinose units. This composition differs significantly from the carbohydrate composition of hot-water extracted Norway spruce wood hemicelluloses where mannose (10% dry wood) and xylose (5%) are the main sugar units (Leppänen et al., 2010). A simple iodine test on the samples revealed the presence of starch. Other hemicelluloses consisting of glucose units, e.g. callose, might also be present and partially explain the high content of glucose in bark. Rather
high amounts of galacturonic acid revealed the presence of pectic substances. Arabinose was also one of the major sugar units and indicated the presence of arabinose-rich hemicelluloses, e.g. arabinans.

**Figure 1.** Carbohydrates composition of the extracted hemicelluloses and pectins during the different steps of extraction

**Molar mass determination**

Molar mass analyses of the three hot water extracts are shown in Table 2. Polysaccharides extracted at 100 °C and 140 °C showed slightly similar molecular mass averages and a rather broad distributions, up to a Mw/Mn of 2.9. The estimated degree of polymerization for these two extracts was around 200. Usually, hemicelluloses extracted from wood have a maximum DP around 200 (Ek et al., 2009) which means that bark hemicelluloses extracted under 140 °C are rather large hemicelluloses. Extraction at 160 °C gave much shorter polymers with a DP of less than 100, which could result from the degradation and hydrolysis of the polysaccharides at high temperature in acidic conditions.

**Table 2.** Alkaline SEC analysis of the three hot water extracts

<table>
<thead>
<tr>
<th></th>
<th>M_n</th>
<th>M_w</th>
<th>Polydispersity</th>
<th>Estimated DP</th>
<th>Peak maxima</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water 100 °C</td>
<td>14240</td>
<td>41300</td>
<td>2.9</td>
<td>230</td>
<td>23800;5700</td>
</tr>
<tr>
<td>Water 140 °C</td>
<td>16240</td>
<td>40640</td>
<td>2.5</td>
<td>230</td>
<td>30500;5800</td>
</tr>
<tr>
<td>Water 160 °C</td>
<td>9400</td>
<td>13510</td>
<td>1.4</td>
<td>80</td>
<td>14000;9600;5500</td>
</tr>
</tbody>
</table>

**Removal of aromatic substances**

We chose to perform the purification step on the fraction from the water extraction at 140 °C. Elution of this aqueous sample through the DAX-8 resin partially removed aromatic substances. The polysaccharide fraction eluted through the resin was freeze-dried and appeared as a colorless powder. A comparison of the 140 °C extract before and after elution through DAX-8 is shown in Table 3. The Klason-lignin content in the fraction significantly decreased to less than 1 % (w/w) and the absorbance at 280 nm also decreased considerably. The UV absorbance of the carbohydrate fraction corresponded to a citrus pectin solution at the same concentration.

**Table 3.** Klason-lignin content and UV absorbance of the water extract at 140 °C before and after elution through DAX-8 resin. The concentration of the solutions for the UV measurements was 0.7 mg/l.

<table>
<thead>
<tr>
<th></th>
<th>Klason-lignin content</th>
<th>Absorbance at 280 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot water extract at 140 °C</td>
<td>9.3 %</td>
<td>0.40</td>
</tr>
<tr>
<td>Carbohydrate fraction from DAX-8</td>
<td>0.9 %</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**IV. CONCLUSIONS**

Almost all hemicelluloses and pectins could be extracted from spruce inner bark with pressurized water at 100-160 °C. Hot-water extraction has environmental and economical benefits and could be applied in industrial practice. Hemicelluloses extracted from spruce bark were very different in composition from those that can be extracted from spruce wood. Arabinose was one of the major sugar units found in spruce inner bark, while mannose is the major sugar units in spruce wood hemicelluloses. In this study, conducted within the European
project PROBARK, we aim to extract, characterize and find new applications for the bark polysaccharides e.g. films, gels or immunostimulating agents.

V. ACKNOWLEDGEMENT
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VI. REFERENCES


