Arabinoxylan and Nanocellulose from a Kilogram-scale Extraction of Barley Husk

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Processes of extracting hemicellulose from annual plants usually neglect the cellulose fraction. This study explored the possibility of obtaining both a hemicellulose and a cellulose fraction of sufficient quality for further use, with barley husks used as the raw material. An alkaline extraction was used to isolate the biopolymers by a process in which sodium dithionite replaced the traditional sodium borohydride as the reducing agent. The cellulose fraction was successfully transformed into nanocellulose by sulfuric acid hydrolysis, and the obtained hemicellulose (*i.e.*, arabinoxylan) displayed carbohydrate composition characteristics similar to those previously reported in the literature for processes of extracting hemicellulose from barley husks. Using this methodology, both the cellulose and hemicellulose can be isolated in high quantities of dry material and used for transformation into new bio-based materials.

Keywords: Hemicellulose; Arabinoxylan; Barley husk; Alkali extraction; Nanocellulose; Cellulose nanocrystals

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INTRODUCTION

From a sustainability perspective, there is a need to replace fossil fuel-based and non-renewable products with biomass equivalents. A common objective is to transform existing pulp mills into biorefineries that can produce various chemicals, therefore obtaining more end uses from the processes. With few exceptions, biochemical platforms primarily convert lignocellulosic biomass into specific products, such as mixed sugars, which are valorized into liquid fuels, propanediol, or polyhydroxybutyric acid. In addition, there is the LignoBoost process to handle the lignin from pulp mills as well as the successful isolation of hemicellulose from pulp process side-streams (Wallmo *et al.* 2009). In kraft pulp mills, the hemicellulose concentration is often below 1% in the black liquor, and membrane filtration allows for kilogram volumes of hemicelluloses to be obtained (Wallberg *et al.* 2006; Persson and Jönsson 2010; Chen *et al.* 2016). The hemicelluloses isolated from pulp process side streams are generally of low molecular weight, making them useful for conversion into fuels and chemicals (Saha 2003).

To obtain high-molecular-weight hemicelluloses, different extraction methods are possible, and alkaline extraction is one of the most common techniques (Höije *et al.* 2005). Alternative, less common methods available includes steam explosion, hot water extraction, or green liquor extraction before pulping (Alvira *et al.* 2010; Jedvert *et al.*

2012; Visuri *et al.* 2012). Different biomass sources contain different types of hemicelluloses with different structure and properties. The most common hemicellulose from hardwood is xylan, whereas glucomannans are the most common hemicelluloses from softwood species. Possible hemicellulose applications include low-oxygen-permeability films for food packaging (Gröndahl *et al.* 2004; Gröndahl and Gatenholm 2007; Mikkonen and Tenkanen 2012) and numerous medical applications (Govers *et al.* 1999; Lu *et al.* 2004; Oliveira *et al.* 2010).

Arabinoxylan (AX) is the most common hemicellulose in annual and perennial plant tissues, with cereal husks containing the highest amount of hemicellulose (Izydorczyk and Dexter 2008). Barley husks, an agricultural by-product, contain approximately 30% to 35% cellulose, 30% to 33% hemicellulose, and 17% to 25% lignin, with the remainder being components such as ash and extractives (Glasser *et al.* 2000; Höije *et al.* 2005; Krawczyk *et al.* 2008; Pitkänen *et al.* 2008; Köhnke *et al.* 2009; Persson *et al.* 2009; Roos *et al.* 2009). The structural features of barley husk arabinoxylan depend not solely on the biomass but also on the extraction method used, and several research groups have explored these structural variations (Eda *et al.* 1976; Shibuya and Iwasaki 1985; Gruppen *et al.* 1992; Saulnier *et al.* 1995; Doner and Hicks 1997; Saha and Bothast 1999; Schooneveld-Bergmans *et al.* 1999; Ebringerová and Heinze 2000; Höije *et al.* 2006; da Silva *et al.* 2012).

By far, the most common process for extracting hemicellulose from annual plants is alkaline extraction (Bergmans *et al.* 1996; Bataillon *et al.* 1998; Gabrielii *et al.* 2000; Höije *et al.* 2005; Deutschmann and Dekker 2012; García *et al.* 2013; Aguedo *et al.* 2014) using sodium hydroxide (NaOH), potassium hydroxide, or barium hydroxide solutions. Alternative methods, such as water extraction (Bengtsson and Åman 1990; Glasser *et al.* 2000), dimethyl sulfoxide extraction (Saake *et al.* 2001), microwave treatment (Lindblad and Albertsson 2004; Roos *et al.* 2009), enzymatic treatment (Faulds *et al.* 2004; Figueroa-Espinoza *et al.* 2004), and extrusion (Vaidya *et al.* 2016), have also been reported. Acid treatments are avoided because they tend to depolymerize the hemicelluloses into monosaccharides and release phenolic compounds (Wallace *et al.* 1995; Aguedo *et al.* 2013). Alkaline treatment of the material causes cellulose swelling, resulting in increased hemicellulose solubility but affecting the native hierarchical structure of the hemicellulose (Izydorczyk *et al.* 1998). However, water extraction of hemicellulose preserves the native hemicellulose structure better than in alkaline solutions, but has the drawback of relatively low yields of isolated hemicellulose (Ebringerová and Heinze 2000). These lower yields are probably a result of lignin carbohydrate complexes, LCCs, that are not cleaved, combined with a low degree of swelling in cellulose, hindering the hemicellulose from becoming soluble in the aqueous phase due to the many hydrogen bonds between the polysaccharides (Takahashi and Koshijima 1988; Maes and Delcour 2001; Zhou *et al.* 2010; Escarnot *et al.* 2011; Giummarella *et al.* 2016; Nishimura *et al.* 2018).

The alkaline isolation of hemicellulose in husks or brans usually follows the sequence: removal of extractives; delignification using sodium hypochlorite, chlorine, or hydrogen peroxide treatments (Maes and Delcour 2001; Hollmann *et al.* 2009); fractionation of hemicellulose from cellulose; and the final precipitation of hemicellulose using alcohols or various salt solutions. Glasser and co-workers (2000) found that pretreatment steps before alkaline extraction, such as pre-hydrolysis and delignification,

resulted in higher purity and lower polydispersity in arabinoxylan from barley husks. In contrast, they also found that the steam explosion treatment of barley husks resulted in severe hemicellulose depolymerization. To purify and concentrate the hemicelluloses in a liquid solution, various filtration and membrane techniques can be used. Most commonly used are dialysis membranes with various molecular weight cut-off values at a laboratory scale, but for upscaling purposes ultrafiltration is an efficient separation method (Krawczyk *et al.* 2008).

One of the earlier reports of hemicellulose isolation and modification at kilogram quantities is a 1995 patent wherein five kilograms of barley husk were extracted and converted into a hydroxypropyl derivative (Glasser *et al.* 1995). Since then, methods for alkaline extraction from cereals have been tested at pilot scale (Faurot *et al.* 1995; Bataillon *et al.* 1998; Hollmann and Lindhauer 2005). Delignification using chlorite on wheat bran has been tried with yields of 13% hemicellulose (Bataillon *et al.* 1998). Pretreatment with green liquor on hardwood chips resulted in hemicellulose extraction of 4% (Johakimu and Andrew 2013).

Unfortunately, when using these processes, there is a lack of affordable bench-scale equipment that can generate hundreds of grams of isolated hemicellulose; consequently, there is a lack of readily available hemicellulose for research groups. To develop a convenient protocol for up to kilogram-scale hemicellulose isolation, a process of alkaline extraction from agricultural crops was explored in this study. The study presented here also highlights the utilization of the side-stream for converting cellulose from barley husk into nanocellulose, according to the biorefinery concept. Almost all articles covering the extraction of hemicellulose from annual plants neglect the cellulose fraction. To obtain both hemicelluloses of high yield and celluloses of sufficient quality to be used in further hydrolysis into nanocellulose, several parts of the method were investigated. As an initial step, the delignification was optimized in a factorial design study focusing on obtaining as much extracted hemicellulose as possible (Claesson *et al.* 2014). In the subsequent alkaline extraction to separate celluloses from hemicelluloses, different reducing agents and alkaline concentrations were evaluated in terms of polysaccharide yield, carbohydrate composition, and cellulose fraction quality. And last, the cellulose obtained after extraction was evaluated as a source for the production of nanocellulose by sulfuric acid hydrolysis.

EXPERIMENTAL

Materials

Milled barley husks (waxy locus of *Hordeum vulgare* L.) were received as a gift from Lyckeby Starch AB (Kristianstad, Sweden). Chemicals used for the extraction process were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received for the following purposes: sodium chlorite ($NaClO_2$) for delignification, sodium borohydride ($NaBH_4$) and sodium dithionite ($Na_2S_2O_4$) for reducing agents, and various concentrations of hydrochloric acid (HCl) and NaOH for pretreatments and alkaline solutions. To reduce the amount of starch in the water soluble fraction, heat-stable α-amylase (Termamyl 120) and rice starch, used for nuclear magnetic resonance (NMR) verification, were also purchased from Sigma-Aldrich. Sulfuric acid (H_2SO_4) was used to

hydrolyze cellulose to nanocellulose, followed by dialysis using Spectra/Por 2 dialysis membrane tubing (MWCO 12-14 kDa) from Spectrum Labs (Rancho Dominguez, CA, USA). Microcrystalline cellulose (MCC, Avicel PH-101) from Sigma-Aldrich was hydrolyzed to nanocellulose for comparison with the nanocellulose obtained from barley husk.

Methods

Delignification

The extraction process was performed using amounts of barley husk (BH) ranging from 10 g to 1 kg per batch. Pretreatment using a 0.05 M HCl solution, 20 mL g^{-1} of BH (*i.e.* 10 L 0.05 M HCl solution to 500 g BH), was done before delignification, to remove extractives from the husks (Glasser *et al.* 2000). The mixture was stirred overnight (16 h) at room temperature, followed by centrifugation to remove the liquid. Delignification was carried out by adding deionized water (4 mL g^{-1} of BH) together with 0.15 g of $NaClO_2$ g^{-1} BH, to the solid material; the pH was adjusted to 3.1 with HCl and the mixture was held at 80 °C for 3 h. This step was optimized in previous work by Claesson *et al.* (2014) in which the pH, amount of $NaClO_2$, and temperature were varied. The delignification reaction was stopped by removing the liquid through centrifugation followed by reslurrying in deionized water and repeated centrifugation. The Klason lignin measured after the delignification step was found to be 11.7%, relative to the barley husk starting material.

Alkaline extraction

The separation between cellulose and hemicellulose was conducted in a 1 M NaOH solution (10 mL g^{-1} BH) containing a reducing agent (0.1 g g^{-1} BH). Two reducing agents were evaluated, the most common one for extractions, $NaBH_4$ and the less toxic and lower-cost alternative $Na_2S_2O_4$. As a reference, extraction without any reducing agent was performed using only a 1 M NaOH solution. The extraction was left overnight (16 h) at room temperature with stirring. The alkali extraction was quenched by neutralization with HCl, resulting in two phases. One water soluble phase, rich in hemicellulose, and one insoluble phase, rich in cellulose. The two fractions were separated through centrifugation, and the solid phase, was washed with deionized water and air-dried for further use. The liquid phase was purified by dropwise addition to a double volume of an ethanol solution (96%), where the hemicellulose precipitated and later separated through centrifugation followed by air-drying.

For the hemicellulose-rich fraction, some glucose was detected by carbohydrate analysis. To evaluate the origin for this glucose units 3 mL of a heat-stable α-amylase was added to 2.5 L solution of the hemicellulosic sample (40 g L^{-1}) and left at 60 °C for 15 hours.

Nanocellulose

The insoluble material obtained in the alkaline extraction, containing a large amount of cellulose, was used to prepare the nanocellulose material. The nanocellulose was produced following a procedure described by Börjesson and co-workers (Börjesson *et al.* 2018). Ten grams of the cellulosic material was added together with 175 mL 64% (w/w) sulfuric acid in a glass beaker and put in a water bath holding 45 °C for 2 h with

gentle shaking. The reaction was quenched by three-fold dilution of deionized water followed by centrifugation at 4300 rpm for 15 min to remove the excess acid and water, to obtain the nanocellulose in the solid fraction. The nanocellulose particles were re-dispersed in deionized water and re-centrifuged. This was repeated twice, followed by dialysis against deionized water until the conductivity was below 5 µS in the dialysis medium. After dialysis, the nanocellulose dispersion was dispersed by sonication at 40% output until a colloidal suspension was achieved. CNCs were also prepared from MCC, which is a common starting material for CNC production, and it was used for comparison with the nanocellulose from barley husk.

Characterization

The neutral carbohydrate composition was analyzed in three replicates on hydrolyzed samples by means of high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), using an ICS 3000 ion chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a CarboPac PA1 (4 mm × 250 mm) analytical column, a gradient pump, an isocratic post-column pump, a column oven, and an autosampler. The sugars detected with the available system were L-arabinose (Ara), D-galactose (Gal), D-glucose (Glc), D-xylose (Xyl), and D-mannose (Man). Before analysis the samples were hydrolyzed with 72% sulfuric acid according to Theander and Westerlund (1986). The hydrolysate was filtered and diluted to 200 g L^{-1}, and the solid residue, the Klason lignin, was gravimetrically analyzed.

Size-exclusion chromatography (SEC) with multi-angle laser light scattering (SEC-RI-MALLS) was used to evaluate the molar mass. This method employs an SEC system consisting of an HPLC pump (Waters, Milford, MA, USA), a Dawn HELEOS II multi-angle laser light scattering detector (Wyatt Technology Co., Goleta, CA, USA), an Optilab T-rEX refractometer (Wyatt Technology Co., Santa Barbara, CA, USA), and a TSKgel GMPWXL column (Tosoh Bioscience, Tokyo, Japan). In this procedure, a solvent of 0.10 mM NaCl and 0.02% sodium azide was used as the mobile phase; the samples were dissolved in the mobile phase at a concentration of 1.0 mg mL^{-1}. Before analysis, the samples were dialyzed against distilled water and filtered through a 0.45-µm syringe filter. The injection volume, flow rate, and temperature were 100 µL, 0.5 mL min^{-1}, and 25 °C, respectively. Pullulan standards (P100, Shodex) were used for internal calibration of the system. The specific refractive index, dn/dc, was measured using a manual injector with the Optilab T-rEX refractometer (Wyatt Technology Co., Santa Barbara, CA, USA). Solutions of different concentrations were used to calculate the dn/dc value of arabinoxylan, which was found to be 0.135. Three replicates of each sample were measured.

Solution-state 2D HSQC NMR measurements were used to detect the α-glucose units from starch in the hemicellulosic samples. The sample was dissolved at 80 °C, and the spectra were recorded in D$_2$O at 25 °C on an Oxford 800 magnet, Avance III HD spectrometer (Bruker, Billerica, MA, USA) equipped with a 5-mm TXO cryoprobe. Two-dimensional HSQC NMR experiments were run using the 'hsqcedetgpsisp2.3' Bruker pulse program with a 1-s relaxation delay, 1000×1000 increments, and eight scans per increment. The acquisition time was set at 0.0799 s. All spectra were processed and analyzed using MestreNova software (Mestrelab Research, version 11.0.0, Santiago de Compostela, Spain).

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Thermogravimetric analysis (TGA) was used to detect thermal changes, such as degradation, in the hemicellulosic samples. A TGA/DSC 3+ Star System (Mettler Toledo, Columbus, OH, USA) was used in which approximately 10 mg of sample was heated from 30 °C to 900 °C under a nitrogen atmosphere at a heating rate of 5 °C min^{-1}, after which the temperature was held at 900 °C for 10 min in air. The onset temperature (T_O) was measured in two replicates using the STARe Software (Mettler Toledo, version 16.00b, Columbus, OH, USA).

Powder X-ray diffraction (XRD) patterns were recorded on a Siemens Diffractometer D5000 (Bruker, Billerica, MA, USA) using a vertical goniometer in Bragg-Brentano geometry and Cu radiation of 45 kV and 40 mA. The crystallinity index (CrI) of the cellulosic material was calculated using the Segal method (Eq. 1) from one measurement of each sample. Because different analytical methods and sample preparation techniques give different crystallinity values, the crystallinity index obtained was used only for the purpose of inter-sample comparison (Terinte *et al.* 2011; Nam *et al.* 2016). Equation 1 is as follows,

$$\text{CrI (\%)} = ((I_{002} - I_a) / I_{002}) \times 100 \tag{1}$$

where I_{002} is the diffraction intensity of the (002) lattice diffraction peak close to $2\theta = 22°$ and represents the crystalline material, and I_a is the diffraction intensity scattered by the amorphous part of the cellulose sample with a diffraction angle close to $2\theta = 18°$.

A scanning electron microscope (SEM) was used to study the morphology of the cellulosic samples. Before SEM analysis, the samples were coated with an approximately 10-nm-thick layer of gold using a JFC-1100E Fine Coat ion sputter (JEOL, Peabody, MA, USA). A LEO Ultra 55 FEG SEM (Zeiss, Oberkochen, Germany) operating at an acceleration voltage of 3 kV was used for the analysis.

Atomic force microscopy (AFM) was used to image the nanocellulose surface topography. Measurements were made using a NanoScope IIIa AFM equipped with a type G scanner (Bruker, Billerica, MA, USA). The measurements were made in tapping mode and in air using an NSC 15 silicon cantilever (MicroMasch, Sofia, Bulgaria). The nanocellulose samples were cast on mica plates from 50 ppm (w/w) solutions.

RESULTS AND DISCUSSION

In this study cellulose and hemicellulose were extracted from barley husks. Figure 1 shows a schematic of the extraction procedure starting at 1000 g milled barley husk with $Na_2S_2O_4$ used as reducing agent. Pretreatment was done with 0.05 M HCl before delignification, to remove extractives from the husks. The delignification was done with $NaClO_2$, where 14% of lignin was removed. The alkaline extraction was performed with 1 M NaOH, and after neutralization the insoluble fraction, *i.e.* cellulose, was obtained (56%). The water soluble fraction was precipitated in ethanol solution, and 25% of hemicellulose was obtained.
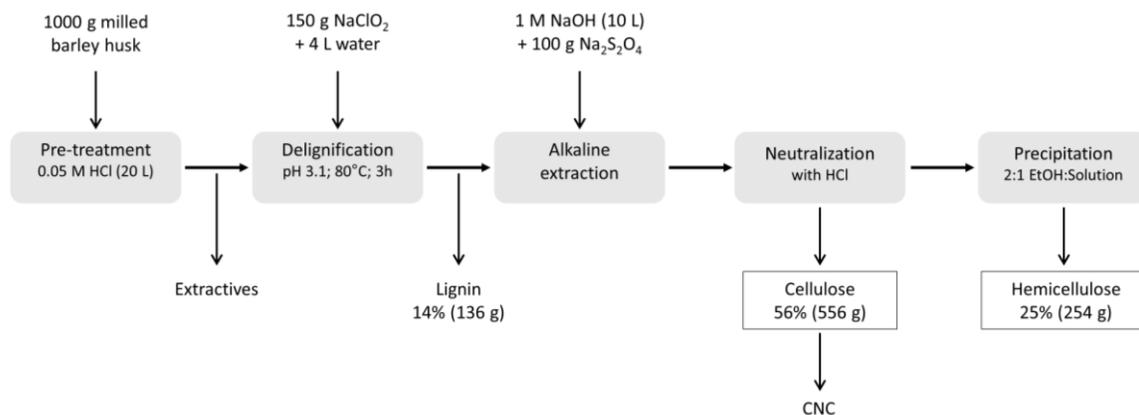
Fig. 1. Schematic of the extraction of cellulose and hemicellulose from barley husks. The extracted cellulose was also collected and evaluated for use as a material source for nanocellulose production. The masses given in the schematic are all in dry weight.

Effect of Reducing Agents

During the alkaline extraction, the polymers can be protected against depolymerization by adding a reducing agent. In small-scale reactions, this is normally done with $NaBH_4$ under alkaline conditions, but it has also been found that $Na_2S_2O_4$ works as an efficient reducing agent for aldehydes (de Vries and Kellog 1980; Wang 2014). During the extraction process it was noticed that the traditionally used $NaBH_4$ created a lot of foam while the neutralization step was performed, which stalled the neutralization, whereas the $Na_2S_2O_4$ caused only moderate foaming. A 1 M NaOH solution was suggested for the extraction. But as it is known that the alkaline treatment of cellulose affects its swelling and disrupts its supramolecular structure and thus crystallinity, a reduction to a 0.1 M NaOH solution was performed in an attempt to obtain a cellulosic fraction of higher crystallinity for nanocellulose preparation. The water-soluble fraction was precipitated in a double volume of ethanol to obtain a solid hemicellulosic material containing several different polymer lengths and arabinose content.

The yields of the different precipitates containing hemicellulosic material were determined by weight. Table 1 indicates that a larger amount of hemicellulose was obtained when using a 1 M NaOH solution and a reducing agent. This corresponded well with the hypothesis that the role of the reducing agent is to decrease or hinder depolymerization. Of the two reducing agents, $NaBH_4$ and $Na_2S_2O_4$, the latter gave a slightly higher yield. The Klason lignin was measured in the different samples (Table 1), which revealed that the sample obtained after extraction with $Na_2S_2O_4$ had a lower lignin content. This result correlated with the findings of Wang (2014), which indicated that lignin is further removed when $Na_2S_2O_4$ is used as the reducing agent.

Table 1. Yield of Extracted Water Soluble Fractions and Klason Lignin in Relation to the Barley Husk Starting Material, Obtained Using Different Reducing Agents or Alkaline Concentrations

Reducing Agent	NaOH Conc. (M)	Yield Water Soluble Fraction (%)	Yield Klason Lignin in Water Soluble Fraction (%)
$NaBH_4$	1	35.3	4.0
$Na_2S_2O_4$	1	37.6	3.1
None	1	22.2	2.9
$Na_2S_2O_4$	0.1	12.2	0.4

The yield is calculated as the weight of water soluble fraction *versu*s the weight of barley husks used from the start. The Klason lignin is obtained from the hydrolysis of the water soluble fraction, relative to the starting material barley husk.

Use of a reducing agent prevents degradation of polymers by protecting the reducing end-groups. The three hemicellulose containing samples extracted using 1 M NaOH solution with different reducing agents, showed difference in color (Fig. 2). The samples without any reducing agent were the darkest in color, while the two samples with reducing agent were more yellow in its color. This darkening in color was attributed mainly to the presence of lignin but may also arise from polymer degradation, which can be induced by heat, oxygen, light, or weathering. Yellowing of polysaccharides is suggested to happen due to decay of hemicelluloses to low molecular carbohydrate compounds that subsequently undergo dehydration and condensation reactions to form colored products (Beyer *et al.* 2006). It may also be due to residues of proteins which can give Maillard-browning (Niquet-Léridon and Tessier 2011).

Fig. 2. Color difference of the hemicellulosic materials prepared with different reducing agents at 1 M NaOH concentration: a) $NaBH_4$ and b) $Na_2S_2O_4$; and c), no reducing agent was used

Analysis of the Water-Soluble Fractions Extracted with 1 M NaOH

Table 2 shows the carbohydrate compositions of the water soluble fractions produced using different reducing agents, extracted with 1 M NaOH. The major carbohydrates present in all hemicellulose rich samples were xylose and arabinose, making arabinoxylan the major hemicellulose present in barley husks. The Ara:Xyl molar ratio was similar in all samples, but a slightly higher arabinose content was found in the sample extracted using $Na_2S_2O_4$, indicated that the $Na_2S_2O_4$ caused less debranching of the arabinose side groups. A carbohydrate analysis of the starting material, barley husks, gave an Ara:Xyl molar ratio of 0.5:1.

Table 2. Carbohydrate Compositions (Relative %) of Hydrolyzed Water Soluble Fractions Obtained after Extraction Using Different Reducing Agents at a Concentration of 1 M NaOH

Reducing Agent	Ara (Rel %)	Gal (Rel %)	Glc (Rel %)	Xyl (Rel %)	Man (Rel %)	Ara:Xyl
$NaBH_4$	26.0	2.6	7.6	63.5	0.3	0.46:1
$Na_2S_2O_4$	26.8	2.7	7.5	62.9	0.2	0.48:1
None	26.4	2.5	6.9	64.1	0.1	0.46:1
The average values of three measurements are listed, and the standard deviation was below 0.2% for all samples. The Ara:Xyl values are presented as molar ratios.						

The carbohydrate analysis detected approximately 7.5% glucose in the hemicellulosic material. To evaluate the origin of this glucose, solution-state 2D HSQC NMR was used to analyze the polymeric samples (Cheng and Neiss 2012); this indicated the presence of α-glucose bonds, demonstrating the presence of starch in the extracted material (Fig. 3). Assignments from the 2D spectra are presented in Table 3. By treating the hemicellulosic material with an α-amylase at 60 °C for 15 h, the glucose content decreased by 77%, as measured by carbohydrate analysis. The remainder of the glucose units in the material originate from β-glucan, cellulose residues, glucose units present in the hemicellulose polymer, or a combination thereof (Höije *et al.* 2005; Persson *et al.* 2009). The addition of α-amylase gave an additional purification step of the extracted water-soluble fraction.
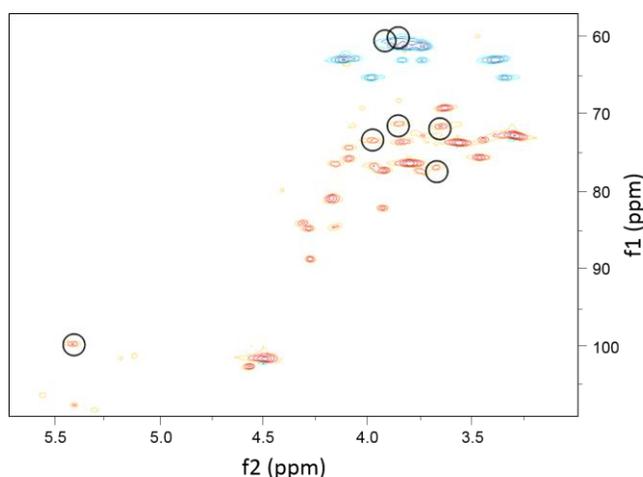

Fig. 3. 2D NMR HSQC experiment performed on the water soluble fraction; the circled peaks are assigned to the starch peaks present in the sample

Table 3. ^{13}C and ^{1}H Chemical Shifts (in Parentheses) for α-Glucose Bonds in Starch in D_2O at 25 °C

Chemical Shifts	C–1 (H–1)	C–2 (H–2)	C–3 (H–3)	C–4 (H–4)	C–5 (H–5)	C–6 (H–6)
^{13}C Shifts	99.63	71.46	71.15	73.34	76.81	60.43
^{1}H Shifts	(5.42)	(3.66)	(3.86)	(3.99)	(3.68)	(3.86) (3.93)

Attempts to determine the molar mass and polymer length of the water soluble polymers, and thus the reducing agents effect on depolymerization was performed with SEC analysis using water based eluent. However, the molar masses obtained from the measurements showed molar masses at 150 to 240 kDa (data not shown), which is not in agreement with previous obtained molar masses on arabinoxylan extracted from barley husk (20-50 kDa) (Höije *et al.* 2005; Köhnke *et al.* 2008; Pitkänen *et al.* 2008; Roos *et al.* 2009). The high molar masses obtained here indicate that the at least some of the hemicellulosic sample was aggregated rather than individual polymers. Thus, together with lignin and/or starch residues, they failed to measure the true molar mass of the individual hemicellulose polymers.

Thermal Stability of Arabinoxylan

Arabinose groups on the xylan backbone have been demonstrated to affect the thermal stability of polymers (Bian *et al.* 2010; Stepan *et al.* 2012). Bian *et al.* (2010) found that AX polymers with a higher arabinose content have a higher decomposition temperature. The decomposition temperatures of the different arabinoxylan materials obtained from the extraction were analyzed using TGA (Fig. 4). The highest onset temperature (T_O) was obtained for the hemicellulosic sample extracted with $Na_2S_2O_4$ (256 °C ± 0.1 °C), which also possessed the highest arabinose content. The lowest value of T_O was obtained for the hemicellulosic material in which no reducing agent was used (240 °C ± 0.1 °C), whereas the sample for which $NaBH_4$ was used as the reducing agent had a T_O of 251 °C ± 0.7 °C. The decomposition temperature is not solely dependent on arabinose content but also affected by parameters, such as polymer length and lignin content.
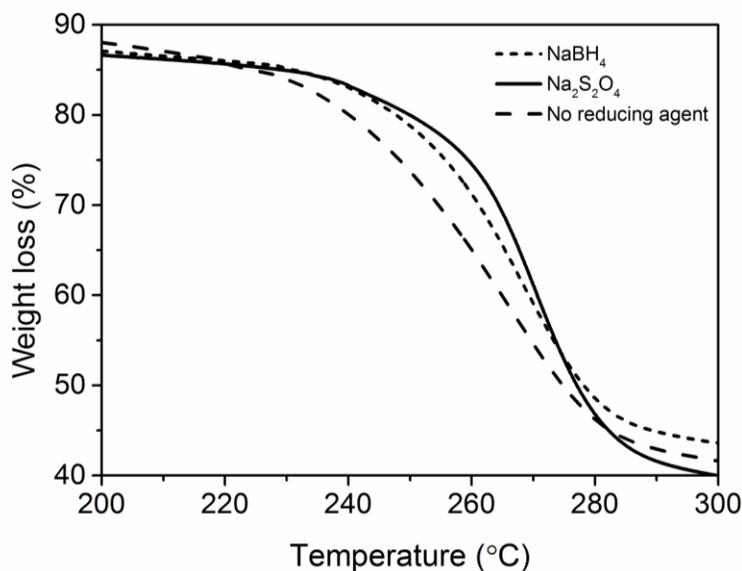

Fig. 4. Partial TGA curves of hemicellulosic samples obtained using different reducing agents in a 1 M NaOH solution; the onset temperatures for the different samples were 251 °C ($NaBH_4$), 256 °C ($Na_2S_2O_4$), and 240 °C (no reducing agent)

Analysis of the Insoluble Fraction

The yields of the different cellulosic containing samples isolated from 1 M NaOH were similar in all samples. However, the lower alkaline solution (0.1 M) gave a larger yield of insoluble material (Table 4), due to the incomplete separation of cellulose and hemicellulose. A carbohydrate analysis of the cellulose rich samples showed residues of arabinoxylan in all samples, but the AX content was higher in the sample obtained from the low alkaline extraction (Table 4). If comparing the Ara:Xyl molar ratio in the hemicelluloses still present in the cellulosic sample, the arabinose content is lower (average Ara:Xyl is 0.27:1) compared to the arabinoxylan obtained in the hemicellulose-rich samples (Table 2, average Ara:Xyl is 0.47:1). This implied that the low-substituted AX had a higher affinity for cellulose than did the high-substituted AX; this is in line with the fact that xylan has a higher affinity for cellulose, whereas higher-substituted AX has a lower affinity for cellulose (Kabel *et al.* 2007).

Table 4. Carbohydrate Compositions (Relative %) of the Hydrolyzed Insoluble Fractions Obtained After Extraction Using Different Reducing Agents and Alkaline Concentrations

Reducing Agent	NaOH Conc. (M)	Yield Insoluble Fraction (%)	Ara (Rel %)	Gal (Rel %)	Glc (Rel %)	Xyl (Rel %)	Man (Rel %)
$NaBH_4$	1	34.6	6.2	2.1	65.9	23.9	1.9
$Na_2S_2O_4$	1	36.8	6.3	2.0	64.2	25.7	1.8
None	1	38.5	6.1	1.9	64.0	26.4	1.6
$Na_2S_2O_4$	0.1	65.7	9.5	2.2	45.0	41.9	1.4

The average values of three measurements are listed, and the standard deviation was below 0.2% for all samples

The cellulosic samples were evaluated with SEM to study their fiber structures. In all samples treated with the 1 M NaOH solution, the fibers were similar in structure, with single fiber fragments as well as bundles of fibers that were not fully separated (Fig. 5). However, there was a large difference between the three samples from a higher alkaline concentration and the sample from the low alkaline concentration. This is apparent in Fig. 5d, which shows large particles without the typical fiber structures, indicating the unsuccessful liberation of the polysaccharides when the extraction was performed with the lower alkaline solution at room temperature.
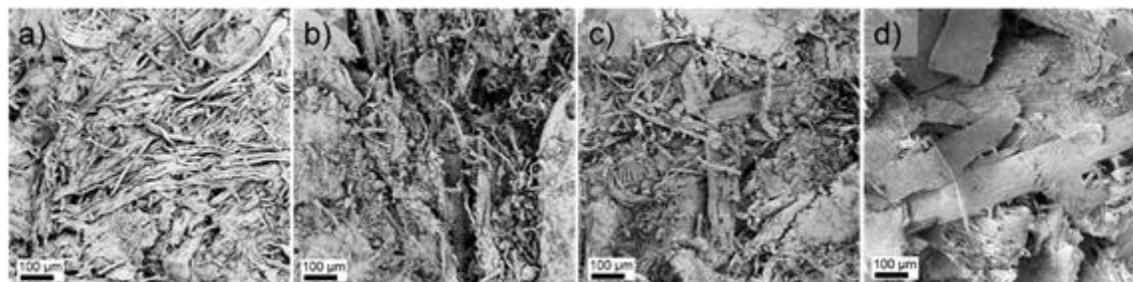

Fig. 5. SEM images of the different cellulosic samples extracted with alkali using different reducing agents and alkali concentrations: a) $NaBH_4$ at 1 M NaOH, b) $Na_2S_2O_4$ at 1 M NaOH, c) no reducing agent at 1 M NaOH, and d) $Na_2S_2O_4$ at 0.1 M NaOH

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Nanocellulose from Barley Husk

To utilize the cellulose containing side-stream from the extraction process, the cellulose samples were hydrolyzed into nanocellulose. Two common material sources for crystalline nanocellulose production are MCC and cotton. Cotton has a crystallinity of approximately 73% and softwood fiber of approximately 65%, whereas MCC (Avicel PH-101), produced by the acid treatment of a cellulose source, has a crystallinity above 88% measured using the Segal method (Terinte *et al.* 2011). One advantage of cellulose from annual plant materials, such as barley husks, is lower price, which may facilitate the development of methods for transforming cereal waste from which hemicellulose and cellulose can be produced into biorefinery products. Table 5 shows the crystallinity of the four cellulosic samples extracted in this study. The hypothesis by using a lower alkali concentration in the extraction process was to preserve more of the crystalline regions of the cellulose fibrils, which could be affected by too much swelling (Zhang *et al.* 2013). The XRD measurements did give a higher crystallinity for the lower alkaline sample compare to the cellulosic samples obtained from extraction at 1 M NaOH with $Na_2S_2O_4$ or without reducing agent. Given the low hemicellulose yield from extraction with 0.1 M NaOH, the slightly higher crystallinity was not enough for it to be considered the recommended procedure.

Table 5. XRD Measurements of the Different Cellulosic Samples Obtained Using Different Reducing Agents and Alkaline Concentrations

Reducing Agent	NaOH Conc. (M)	Crystallinity (%)
$NaBH_4$	1	69.1
$Na_2S_2O_4$	1	61.7
None	1	63.6
$Na_2S_2O_4$	0.1	69.3

No replicates were measured.

The procedure for producing nanocellulose was as described earlier, and the cellulosic samples used for the hydrolysis were isolated from 500 g barley husks where $Na_2S_2O_4$ was chosen as the reducing agent. A batch was prepared from MCC as a reference sample, and the two nanocellulose samples were analyzed using AFM to study the crystal topography. Figure 6 shows the nanocellulose samples from the MCC and the barley husk cellulose. The sample prepared from the barley husk cellulose had a more fibril-like structure compare to the rod-like structures of CNC prepared from MCC, though still with a diameter in the nanometer range. However, it is known that different cellulose sources give different nanocrystal sizes and shapes (Börjesson and Westman 2015). Espino *et al.* (2014) evaluated nanocellulose prepared from barley husks using a similar hydrolysis process, and they also concluded that barley husk nanocellulose gives longer fibrils with a higher aspect ratio than does CNCs from MCC. In general, it is suggested that nanocellulose can be used for reinforcement in renewable nanomaterials. The high aspect ratio obtained for nanocellulose from agricultural wastes could therefore be beneficial in biocomposite manufacturing.
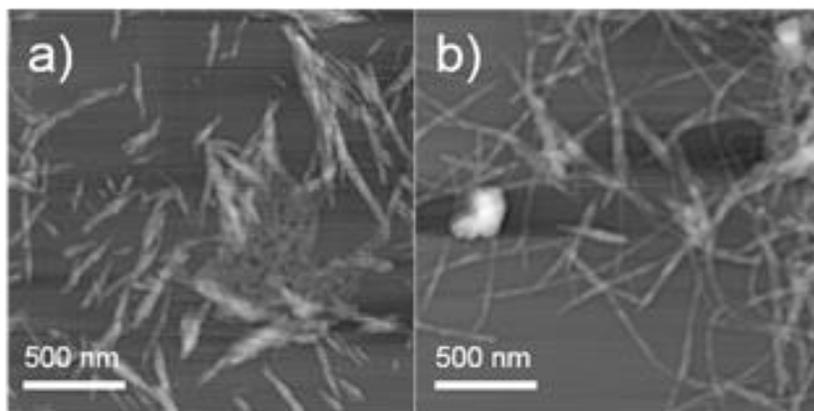
Fig. 6. AFM images of nanocellulose prepared using sulfuric acid hydrolysis from a) MCC and b) barley husk cellulose

Protocol for Extractions from Barley Husk

From the delignification and extraction studies, reaction parameters were determined to obtain high content of hemicellulosic materials after completed extraction. Reaction steps used for an extraction of 1 kg quantities of barley husk:

(1) Prehydrolysis using a 0.05 M HCl-solution (20 mL g^{-1} BH) over night.

(2) Removal of extractives through centrifugation followed by $NaClO_2$-delignification using 0.15 g $NaClO_2$ g^{-1} BH in water (4 mL g^{-1} BH). pH adjustments to 3.1 with HCl. Delignification occurred at 80°C during 3 h.

(3) Separation of the lignin fraction through centrifugation, followed by extraction with a 1 M NaOH-solution (10 mL g^{-1} BH) and with addition of 0.1 g $Na_2S_2O_4$ g^{-1} BH over night with continuous stirring.

(4) To separate the cellulose from the hemicellulose, the solution was neutralized with HCl in order to obtain the cellulose containing part as a solid product and the hemi-cellulose containing part in the liquid phase.

(5) Finally, the water soluble fraction rich in arabinoxylan was precipitated in ethanol (2:1 volume ratio) and collected as a solid material after removal of the liquids. Different drying techniques such as freeze drying or spray drying can be used.

CONCLUSIONS

This study developed a convenient protocol for research groups to extract hemicelluloses in large enough quantities for laboratory work.

1. The results of this study indicated that $Na_2S_2O_4$ can be used instead of traditional $NaBH_4$ as the reducing agent, giving a slightly higher arabinose content and thus increased thermal stability and water solubility. Other advantages with replacing $NaBH_4$ with $Na_2S_2O_4$ are reduced toxicity and lower costs. Additionally, replacement of $NaBH_4$ with $Na_2S_2O_4$ led to more effective handling during the neutralization step of the extraction process, since the foaming during the neutralization step was

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significantly reduced. A positive effect on hemicellulose discoloring due to further removal of lignin from the samples was also achieved.

2. High quantities of the hemicellulose arabinoxylan were obtained. With this convenient protocol developed here, approximately 250 g of dried arabinoxylan was obtained from a starting material of 1 kg barley husk. The only impediment for further scaling-up was due to the size of the lab equipment.

3. The extracted cellulosic material could serve as a source for CNC production. The crystallinity of the extracted cellulose was similar to that of wood fiber. The nanocrystals formed were larger than the nanocrystals from MCC hydrolyzed using the same method, but the larger crystals gave a higher aspect ratio that could be beneficial in, for example, biocomposites.

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