

Pressurized Hot Water Flow-through Extraction of Birch Sawdust with Acetate pH Buffer

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Birch sawdust was extracted with a pressurized hot water (PHW) flow-through system. The pH of the extract was controlled during extraction by the addition of acetic acid/sodium acetate buffer. Three different pH levels, 4.0, 4.2, and 4.6, were used. Extraction temperatures were 160, 170, and 180 °C, with a constant 4 mL/min flow rate through the 50 mL extraction vessel. During PHW extraction with plain water, the end pH of the extract dropped from 5 to below 3. Buffers kept the pH of the extracts constant during the first 30 min of extraction. Compared to plain water, the use of buffers resulted in a lower yield of xylans during the 170 °C extraction. However, with the buffered system it was possible to obtain xylans from sawdust with a higher molar mass than plain water extraction. Acetic acid/sodium acetate buffer enabled control of the hydrolysis rate of xylans during extraction.

Keywords: Pressurized hot water extraction; Flow-through; pH buffer; Birch; Sawdust; Xylan

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INTRODUCTION

Dwindling fossil fuel supplies and toughening carbon dioxide legislation are driving forces for the use of renewable resources (Ragauskas *et al.* 2006). Of these, wood is a suitable raw material source for many processes. It is a renewable resource that does not require large amounts of fertilizers or insecticides during growth, as it is able to grow by itself (Liu 2010). Hemicelluloses are the third most abundant compound in wood (Sjöström 1993). However, wood hemicelluloses are not yet fully industrially utilized. Birch is a hardwood growing in the northern hemisphere in temperate and boreal climates and is currently used in sawmills and for kraft pulping. Additionally, water is an environmentally friendly solvent and is widely used in industry. Pressurized hot water can be used to isolate xylans, the main hardwood hemicellulose, from birch sawdust for various high-value applications. Xylans have the potential to be used in biomedical applications (Ebringerová and Hromádková 1999), to produce composite films with cellulose (Saxena *et al.* 2011), as food additives and nutraceuticals (Moure *et al.* 2006), as food packaging films (Mikkonen and Tenkanen 2012), and as foams or gels (Deutchmann and Dekker 2012). Xylan can also be chemically functionalized further to yield cationic xylans (Schwikal *et al.* 2005) or to increase the size of xylan molecules with reductive amination (Daus *et al.* 2010).

Many hemicellulose extractions from wood are performed with water at elevated temperatures and under pressure, in order to keep water in liquid form to solvate the polymers. A pressurised hot water batch system has recently been used to extract birch

sawdust (Borrega *et al.* 2011a; Borrega *et al.* 2011b). Maple, aspen, and birch chips were pre-hydrolysed using a similar system (Li *et al.* 2011) to remove xylose and xylo-oligosaccharides. Birch chips were also treated in a batch reactor (Testova *et al.* 2011) at 150 and 170 °C to extract xylans. Hardwood chips were extracted using a continuously mixed batch reactor to extract xylans by Chen *et al.* (2010). Al Dajani and Tschirner (2010) have compared alkaline pre-extraction and autohydrolysis for pulping. In similar conditions, alkaline pre-extraction led to better pulp, but autohydrolysis gave a higher yield of extracted hemicelluloses.

Pressurized hot water extraction has an effect on the extracted material. The surface characteristics of PHW-extracted red maple strands were studied by Paredes *et al.* (2009). The porosity and the crystallinity of the extracted strands increased. Also the width of the cellulose increased after PHW extraction. Pine flakes were extracted with PHW before they were blended with phenol-formaldehyde resin and wax emulsion to make a flakeboard panel (Hosseinaei *et al.* 2011a). The hydroscopicity of panels made of PHW-extracted flakes decreased and panels had better dimensional stability and mould resistance than other panels. Pine strands were PHW-extracted in similar conditions to remove hemicelluloses (Hosseinaei *et al.* 2011b). The PHW extraction increased the hydrophobicity of wood strands due to the removal of the hemicelluloses. PHW-extracted wood flour was compounded with polypropene to make composites (Hosseinaei *et al.* 2012). Water absorption of the composites decreased and tensile properties of the composites increased.

Thermohydrolysis using flow-through PHW has been used for biomass fractionation (Bobleter and Pape 1964; Bonn and Bobleter 1983; Bobleter 1994; Bobleter *et al.* 1976). Polymeric and oligomeric xylan were recently extracted from birch sawdust using a pressurized hot water flow-through system (Kilpeläinen *et al.* 2012). This method is also suitable for softwoods such as spruce (Leppänen *et al.* 2011). Almost all hemicelluloses from spruce sawdust could be extracted at 200 °C. In addition, flow-through extraction has been used to extract corn stover (Liu and Wyman 2004). When the authors compared extraction using a batch reactor and a flow-through system, the flow-through reactor resulted in higher xylose yield, greater lignin removal, and less extensive hydrolysis and breakdown of hemicelluloses.

Recently, there have been results published in which the pH of the batch extractions of spruce galactoglucomannan was controlled using different buffers, such as sodium bicarbonate (Song *et al.* 2011a) and phthalates (Song *et al.* 2011b). The yields of the extractions were similar to those with plain water extraction. Buffers decreased the hydrolysis of hemicelluloses and prevented deacetylation of softwood galactoglucomannans.

Usually only the temperature and reaction time are controlled during autohydrolysis. The developing acidity during extraction is a result of carboxylic acid groups, which are released by the hydrolysis of esters during extraction. The amount of acidic groups in wood and other biomass varies between species and even within the same species.

The overall aim of this study was to control the pH of the media during hemicellulose extraction of birch sawdust, using buffers. The goal was to decrease the hydrolytic cleavage of xylans during PHW flow-through extraction and to obtain higher molar mass hemicelluloses after the extraction with pH buffer.

EXPERIMENTAL

A twenty-year-old birch tree (*Betula pendula*) was felled and sawn to sawdust at the Finnish Forest Research Institute research forest at Ruotsinkylä in southern Finland. The birch sawdust was stored at -20 °C in the dark and in sealed bags. The sawdust was freeze-dried to a constant weight before extractions to dry samples before storage. The birch sawdust contained different sized particles; therefore, the particle size distribution was determined using differently sized sieves (Retch, Germany) and is shown in Table 1.

Table 1. Birch Sawdust Particle Size Distribution

Size of sawdust (mm)	Sawdust weight (wt%)
> 2.0	1
2.0-0.63	28
0.63-0.2	53
0.2-0.063	18
< 0.063	0

The amounts of hemicelluloses, lignin, and cellulose were determined previously for the same sawdust batch (Kilpeläinen *et al.* 2012). The birch sawdust contained 40% cellulose, 25% hemicelluloses, 27% lignin, and 2% of extractives. The main hemicellulose sugar content was 16.6% (of dry wood) xylose, 2.1% glucose, 2.0% 4-*O*-me-glucuronic acid, and minor amounts of other sugars.

Extraction Set-up

Extractions were performed with a laboratory-built PHW flow-through extraction system. Details of the extraction set-up are described in a previous paper (Kilpeläinen *et al.* 2012). About 10 g of birch sawdust were placed in a 50 mL extraction vessel and extracted with PHW using the flow-through system with plain water or three different sodium acetate/acetic acid buffers at pH 4.0, 4.2, and 4.6. Extractions were performed at three different temperatures 160 °C, 170 °C, and 180 °C using a 4 mL/min flow for 30 min extractions. Ten-minute fractions of the extract were collected in volumetric flasks during extraction. The temperature inside the extraction vessel was measured using a laboratory-made 50 mL extraction vessel. The vessel had an inlet where a thermocouple could be placed and 10 g sawdust loading was used in these extractions.

Buffer

Sodium acetate/acetic acid buffer was used to control the pH during and after extraction. An acetic acid buffer was chosen because acetic acid is released during PHW extraction, so no new chemical would be added to the extraction system. Acetic acid could also be recycled after extraction.

Three different pH values, 4.0, 4.2, and 4.6, were chosen for the extraction. The buffer strength was 0.1 M at buffer pH 4.0 and 4.2. The buffer strength was 0.05 M at pH 4.6. The aim was to use as little buffer as possible to keep pH values near 4 to prevent excessive autohydrolysis. Increasing the buffer strength keeps the pH more stable during extraction, but it also introduces more sodium into the extraction system. The pH values of the buffers were adjusted to the designated value with sodium acetate or acetic acid after dilution.

Residence Time

The residence time of water inside the flow-through system is a factor for determining the contact time of water and sawdust during extraction. The residence time of water inside the extraction vessel was calculated using Eq. 1,

$$t = \frac{V_{\text{vessel}} - V_{\text{sawdust}}}{q} \quad (1)$$

where t is the residence time (min), V_{vessel} is the volume of the extraction vessel (mL), V_{sawdust} is the volume of sawdust solids (mL), and q is the flow through the extraction vessel (mL/min). When water flows through the empty extraction vessel, the residence time is 12.5 min. By adding sawdust, the volume inside the extraction vessel decreases and therefore the residence time is shorter. Since wood is a porous material, it takes a larger volume amount than more dense particles. By using the density of wood solids 1.530 g/mL (Sixta 2006) for sawdust in calculations and 10 g of air-dry sawdust, sawdust solids takes 12% of the extraction vessel volume (V_{sawdust}) and the residence time (t) of water inside the extraction vessel is 11.0 min. The constant flow of hot water dissolves hemicelluloses and lignin from the wood matrix and moves them out of the extraction vessel (Kilpeläinen *et al.* 2012). The rate of hydrolysis is less severe than with a batch reactor, but more water is used compared to batch reactor extractions (Liu and Wyman 2004).

Severity of Extractions

The severity of the extraction varies with different kinds of hot water extraction systems. Batch reactors have the extracted material inside the reactor for longer times. Thus, extract and extraction residue have essentially the same treatment time. However, with a flow-through vessel, extract and extraction residue can have different treatment times. Extract flows out of the vessel continuously during treatment and can have lower extraction severity than the birch sawdust, which is inside the vessel for the whole treatment time. This difference is not significant with short extraction times but it can have a stronger effect with longer extraction times. With longer extractions than demonstrated here, severity factors should be calculated separately for extracts and extraction residues.

Severity factors are used to make comparisons between different kinds of extraction systems and calculate the effect of extraction on the extraction residue. The severity of the extractions can be calculated using different severity factors. The prehydrolysis factor (P-factor) was introduced by Brasch and Free (1965). The severity factor R_0 is calculated using Eq. 2,

$$R_0 = \int_{t_0}^t \exp\left(\frac{T(t)-100}{14.75}\right) dt \quad (2)$$

where t is the treatment time in minutes and $T(t)$ is the extraction temperature. Overend *et al.* (1987) used a logarithm of severity factor R_0 that is also used in literature to measure the severity of the extraction. In a recent publication, more detailed information about activation energy (A_e) of xylan hydrolysis was determined, and the P-factor was calculated using an activation energy of 125.6 KJ/mol, for fast hydrolysing xylan (Sixta 2006), resulting in Eq. 3,

$$P = \int_{t_0}^t \exp\left(40.48 - \frac{15106}{T}\right) dt \quad (3)$$

where P is the P-factor (prehydrolysis factor), t is time (h), and T is the extraction temperature (K). Both severity factors use 100 °C as a reference reaction rate temperature for xylan hydrolysis. However, severity factor R_0 and P-factor have limitations on their usefulness, since pH values during extraction are not accounted for. Pedersen and Meyer (2010) used pH to calculate the severity of pretreatments. The combined severity factor ($\log(R'')$) was used to calculate the severity of pretreatment for different substrates, ranging from wheat straw to softwood and hardwood. In addition, different pretreatment methods were compared, including acidic steam explosion (ASE), alkali wet oxidation (AWO), and lime pretreatment, using wheat straw as a substrate. The combined severity factor has also been used to describe the substrate's accessibility to following enzymatic treatments. The combined severity factor is determined using Eq. 4,

$$\log(R'') = \log R_0 + |pH - 7| \quad (4)$$

where R_0 is the severity factor from Eq. 2.

A similar equation can be formed from the P-factor with pH using Eqs. 3 and 4,

$$\log(P') = \log P + |pH - 7| \quad (5)$$

where $\log(P')$ is the modified P-factor and P is the P-factor.

Analysis of Extract

Extracts were diluted to a constant volume in volumetric flasks before analysis. Extracts were stored in the freezer (-20 °C) in cases when analyses were not performed directly after extraction. The amount of extracted xylan was determined with GC-FID following acid methanolysis (Willför *et al.* 2009). Depending on the concentration, 0.5 to 5 mL of extract was frozen (-20 °C) and then freeze-dried. Two calibration solutions with equal amounts of analysed monomeric sugars; glucose, galactose, mannose, rhamnose, xylose, arabinose, glucuronic acid, and galacturonic acid were used. Calibration solutions were used to calculate the response factor for each sugar, except 4-*O*-MeGlcA, which response factor was calculated from glucuronic acid. Samples and calibration solutions were treated similarly during analysis. Samples were depolymerised with acid methanolysis, using 2 mL of 2 M HCl in anhydrous methanol and they were kept at 100 °C for 3 h. Samples were cooled to room temperature and were neutralised with 200 µL pyridine. After neutralisation, 1 mL of methanol solution containing 0.1 mg/mL of sorbitol and 0.1 mg/mL of resorcinol were added as internal standards to the samples. Samples were dried under a nitrogen stream and were further dried for 15 min in a vacuum oven at 40 °C to remove any remaining moisture from the samples. Samples were then silylated by adding 100 µL of pyridine, 150 µL of hexamethyldisilazane (HMDS), and 70 µL of trimethylchlorosilane (TMCS). Samples were silylated overnight and were analysed using GC-FID. The results were reported as amounts of anhydrosugars by multiplying glucose, mannose, and galactose by 0.9; arabinose and xylose by 0.88; rhamnose by 0.89; and glucuronic acid, galacturonic acid, and 4-*O*-methylglucuronic acid by 0.91.

Monosaccharides were analysed from freeze-dried aliquots. GC analysis was performed on silylated samples using xylitol as an internal standard. The amount of fructose in the extracts was calculated by multiplying the results by a calibration factor of 0.7. GC analyses were performed using a Shimadzu GC-2010 (Shimadzu, Kyoto, Japan) gas chromatograph, equipped with a HP-1 column (25 m, 0.2 mm, 0.11 μm). The relative amount of oligo- and polysaccharides compared to monosaccharides were calculated by reducing amount of monosaccharides from acid methanolysis results.

The total dissolved solids (TDS) was determined using a Kern lb 535 moisture analyzer. Dissolved solids were obtained from 5 g of extract at a temperature of 138 °C. The average molar mass (Mw) of extracted hemicelluloses in the extracts was determined using a HP-SEC with a refractive index detector (RID) (Shimadzu Corp., Tokyo, Japan) and a multi-angle laser scattering (MALLS) detector (miniDAWN, Wyatt Technology, Santa Barbara, CA, USA). The HP-SEC system had a guard column (Ultrahydrogel 6 mm \times 40 mm; Waters, Milford, MA, USA) and two main columns (UltrahydrogelTM linear 8.9 mm \times 300 mm; Water, Milford, MA, USA) connected in series. A dn/dc value of 0.15 mL/g for xylan was used for molar mass calculations.

Lignin content was determined by UV spectrophotometry after removing lignans and other extractives by methyl-tert-butyl ether (MTBE) extraction of the water extracts, according to Örså *et al.* (1997). After extraction, a Shimadzu UV-2401PC (Shimadzu, Kyoto, Japan) spectrophotometer was used to measure the absorbance of samples at 280 nm. An extinction coefficient of 49 mg cm L⁻¹ was used to calculate the amount of lignin-related substances.

Analysis of Wood

All calculations for wood were determined using dry wood. The amount of hemicelluloses in the wood raw material and extracted sawdust were determined by acid methanolysis of the dried wood for 5 h and silylation followed by gas chromatography. This procedure was the same as the analysis of hemicelluloses in extracts (Willför *et al.* 2009).

RESULTS AND DISCUSSION

Extract pH

Sawdust was extracted with plain PHW or with buffer. During extraction with water, the pH decreased from 5.7 to 3.7 at 160 °C (Fig. 1a). Sodium acetate buffer kept the pH of the extracts relatively stable during the extractions. Three different buffers were used with pH values of 4.0, 4.2, and 4.6. At all temperatures, the initial pH of the buffered extractions was lower than for plain water. At 170 °C, the pH of the water extractions decreased to 2.9 after 20 to 30 min extraction, while the buffered extracts maintained a higher pH. In the 180 °C extraction, the pH decreased in a similar manner as the 170 °C extraction using plain water, while the pH of the buffered systems only decreased a little (Fig. 1b).

The pH at 160 °C decreased two units, from 5.8 to 3.8, at the end of the extraction with plain water. During the pH-buffered extraction, the pH of the extract decreased slightly. The largest pH decrease was 0.5 units with pH 4.6 buffer. At 160 °C, the pH was lower with the buffer at first 20 minutes of extraction than with plain water. Chen *et al.* (2010) used a continuous mixed batch reactor with hardwood chips and acetic acid at pH

3.3 and 3.8. In their experiments, the pH was constant during the extraction and the pH decreased similarly with plain water.

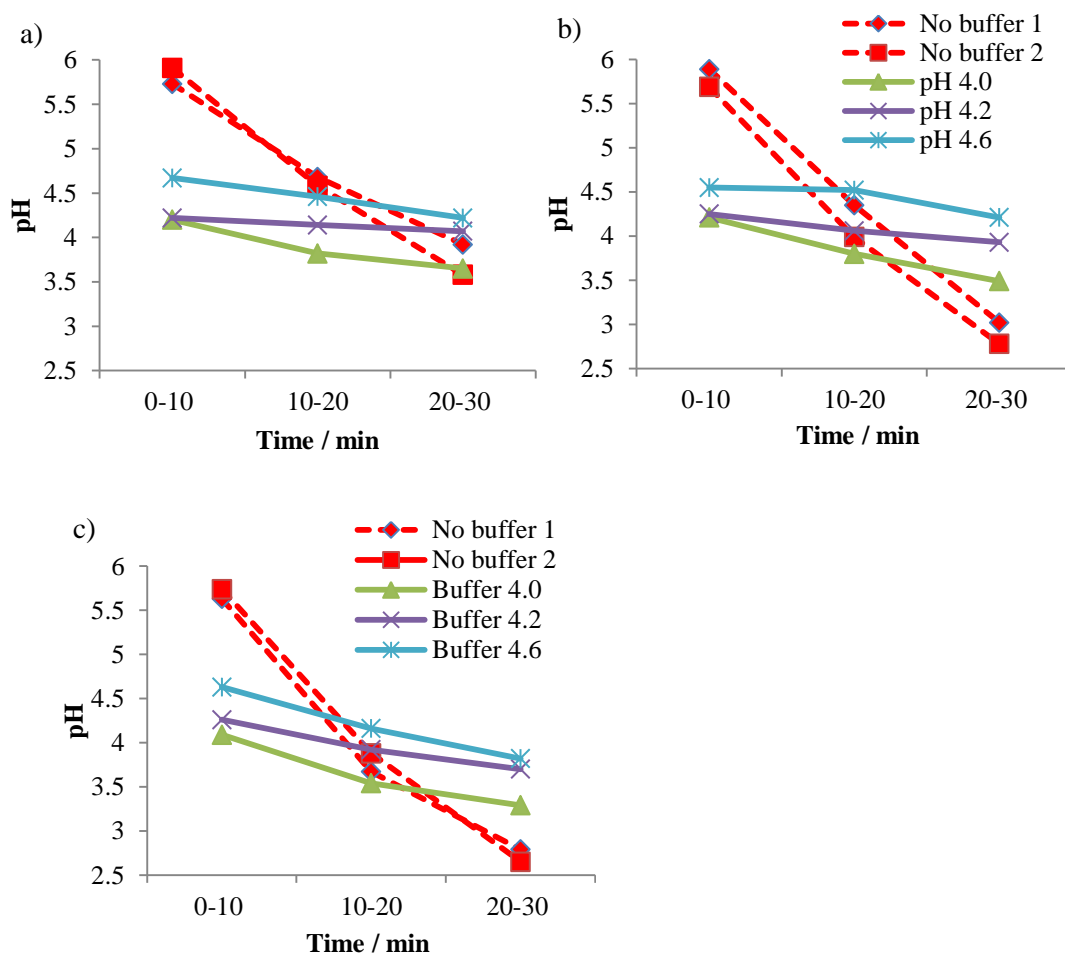


Fig. 1. The end pH of the extracts after (a) 160 °C, (b) 170 °C, (c) 180 °C PHW extractions

The end pH of the water extracts at 180 °C decreased to 2.7, which is 0.2 pH units lower than at 170 °C (Fig. 1(c)). The higher extraction temperature at 180 °C probably liberated more acetic acid from hemicellulose chains and released more uronic acids from pectins. Even at 180 °C, buffers kept the pH of the extract relatively stable compared to plain water, although a clear drop was evident during treatment. Using the pH 4.0 buffer, the pH decreased only 0.8 pH units during the 30 min extraction. Similar pH control has been obtained with Norway spruce using 0.1 M NaHCO₃, giving a starting pH of 8 (Song *et al.* 2011a) and with Norway spruce using 0.1 M phthalate buffers, giving starting pH values of 3.8, 4.0, 4.2, and 4.4 (Song *et al.* 2011b). During plain water extractions, the pH values decreased up to 1.8 pH units at 160 °C and maximum 3.1 units at 180 °C. When buffers were used, the pH decreased less than 0.15 pH units at a PHW extraction temperature of 160 °C and 0.8 pH units at 180 °C. The differences in pH are significant, since the pH scale is logarithmic and control of the pH during extraction adjusts the rate of xylan hydrolysis during extraction.

Total Dissolved Solids

The amount of total dissolved solids (TDS) increased at higher extraction temperatures (Fig. 2).

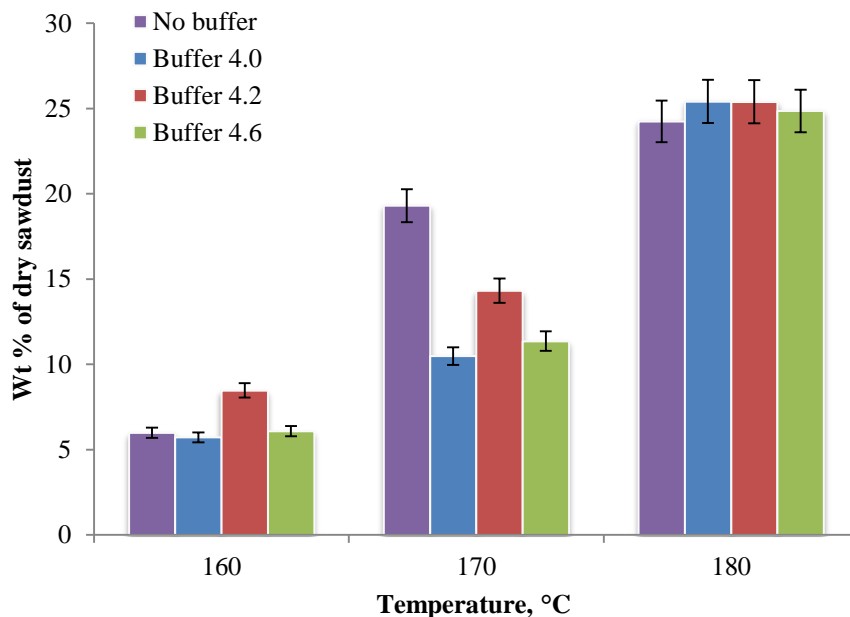


Fig. 2. The sum of total dissolved solids of the PHW extracts at different extraction temperatures. Error bars are for 5% relative standard deviation.

The amount of TDS was similar for both plain water and buffer extractions, except at 170 °C. At 170 °C, the buffered solutions extracted a smaller amount of solids than plain water extractions. When extractions at 170 °C were compared against extraction time, buffered extractions had lower TDS at 20 to 30 min fractions than water extractions. Inhibition of hydrolysis could prevent the dissolution of xylan. At 170 °C, most of the birch material would be released after 20 to 30 min when buffers were used. With buffers, the highest concentration of extracted material would occur at 30 to 40 minutes. Therefore, total dissolved solids were lower in the pH-buffered extraction compared to the plain water extraction. At 180 °C, the combination of temperature and pH was enough to release the same amount of material from birch sawdust in both plain water and buffered extractions.

Extracted Carbohydrates

The amount of extracted xylans from birch sawdust is shown in Fig. 3. Similar to the TDS results, the highest yield of carbohydrates was obtained at 180 °C. The amount of extracted carbohydrates was also different when plain water or buffered solutions were used. The yield with plain water was higher at 170 °C than the buffered solutions. The addition of buffer probably lowered the extraction yield by inhibiting hydrolysis, as was observed with Norway spruce extractions using the ASE batch system (Song *et al.* 2011a,b) at 170 °C with sodium bicarbonate and phthalate. The extract pH is an important parameter during extractions, but the temperature of the extract must be high enough to release xylan from the birch sawdust (Fig. 3).

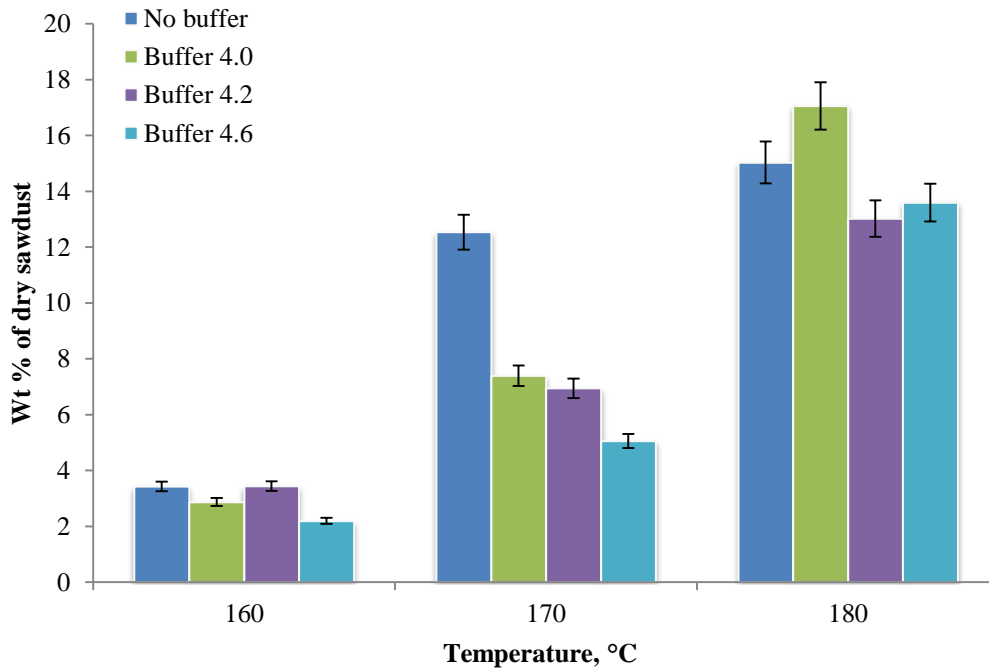


Fig. 3. PHW-extracted non-cellulosic carbohydrates at different extraction temperatures. Error bars are 5 % relative standard deviation from birch sawdust determination

The sugar compositions for the extracts were similar for all buffers; pH 4.0 was selected for comparison with plain water (Fig. 4). Yields and compositions of the different carbohydrates in oligo- and polysaccharides were similar in both extraction series, except for the yield at 170 °C for 20 to 30 min extraction time.

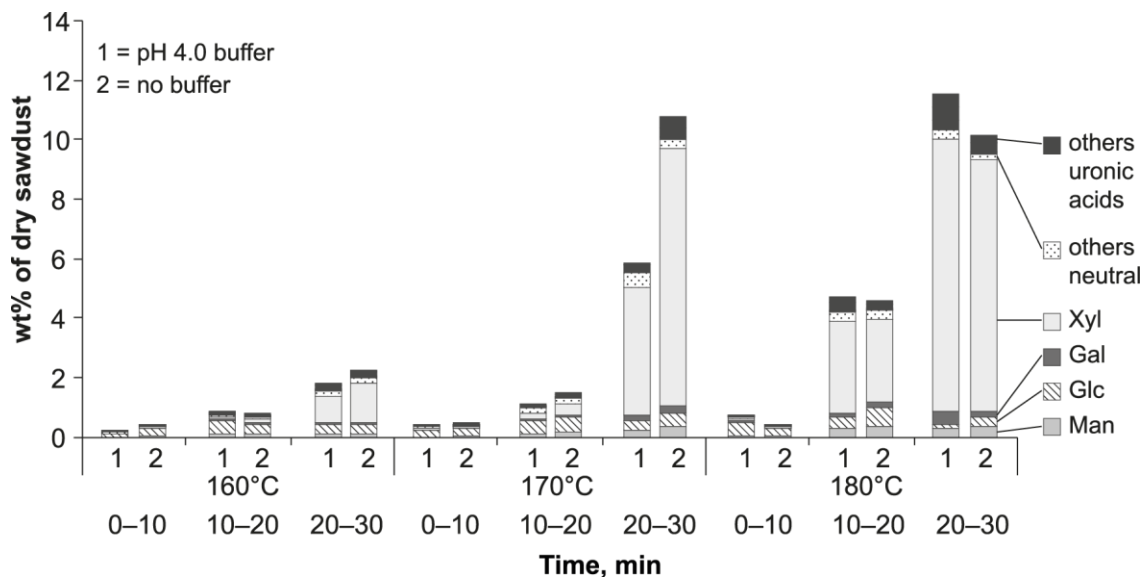


Fig. 4. PHW extractions with pH 4.0 buffer and without buffer

Extracted Lignin

Lignin was analysed from the PHW extracts by UV absorption after MTBE extraction (Table 2).

Table 2. Extracted Lignin

Temperature, °C	wt% lignin of dry wood			
	No buffer	pH 4.0	pH 4.2	pH 4.6
160	0.8	0.7	0.8	0.7
170	1.4	0.5	1.1	1.1
180	1.6	1.4	2.1	2.1

The highest lignin yield was obtained at 180 °C with and without pH buffer. PHW extraction can degrade the lignin from birch sawdust to lignin-related substances. UV spectrometric determinations in this work used an extraction coefficient that was obtained from the measurement of milled wood lignin (Kilpeläinen *et al.* 2012). The extraction coefficient for lignin-related substances would probably be different. To obtain more accurate results for lignin-related substances with this method, a new extraction coefficient should be measured. However, the results in Table 2 show that more lignin-related substances are extracted from sawdust as the extraction temperature increases.

Amount of Hemicelluloses and Monosaccharides

During extraction, part of the xylan was hydrolysed to monosaccharides. The ratio of monosaccharides vs. extracted oligo- and polysaccharides gives an indication of the severity of hydrolysis. Xylose and arabinose from xylan can further react to form furfurals. Furfurals can further react with xylose intermediates and polymerize to condensates and resins (Sixta 2006; Zeitch 2000). The amount of extracted carbohydrates at 160 °C is shown in Fig. 5.

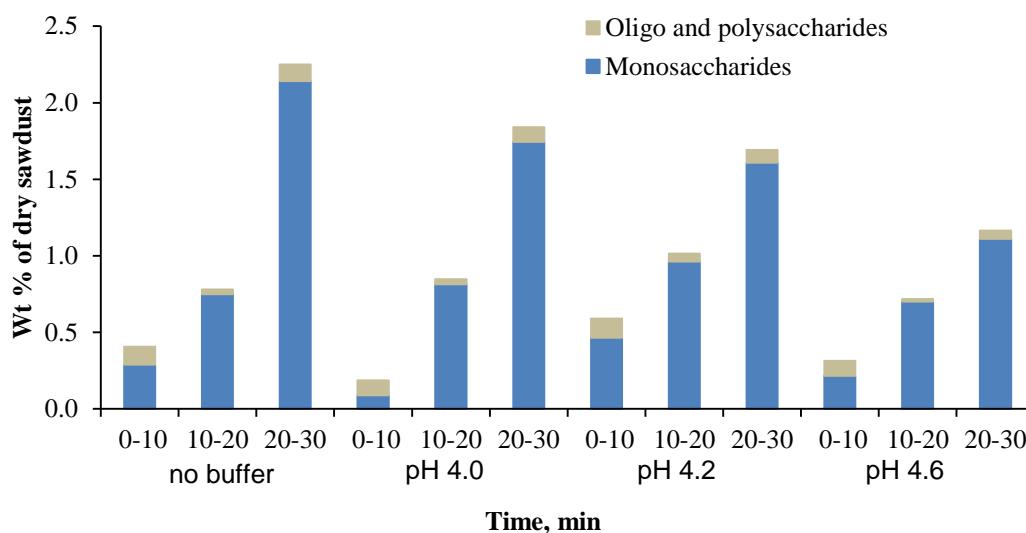


Fig. 5. The amount of extracted mono, oligo-, and polysaccharides at 160 °C extraction with PHW and buffered extractions

There were more monosaccharides released at the beginning of the extractions from 0 to 10 min. Most of the extracted monosaccharides were easily solvated sugars, like glucose and fructose, which could arise from starch or saccharose. Yields of extraction at 160 °C were low, only 2 to 4% of sawdust was extracted, which was 8 to 16% of the xylans in sawdust.

At the higher temperatures of 170 °C and 180 °C, more hemicelluloses were extracted from birch sawdust (Fig. 6 and 7).

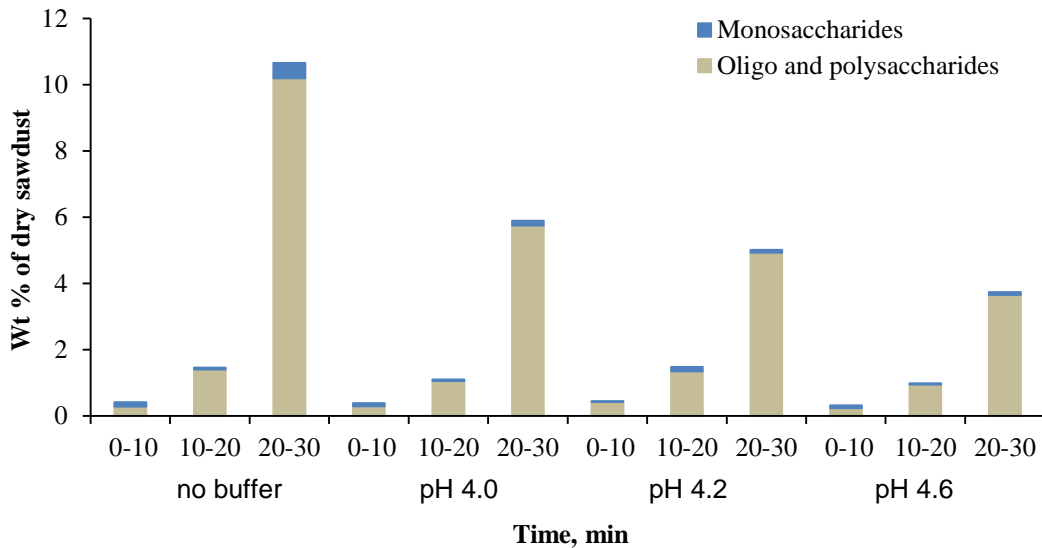


Fig. 6. The amount of extracted mono-, oligo-, and polysaccharides at 170 °C

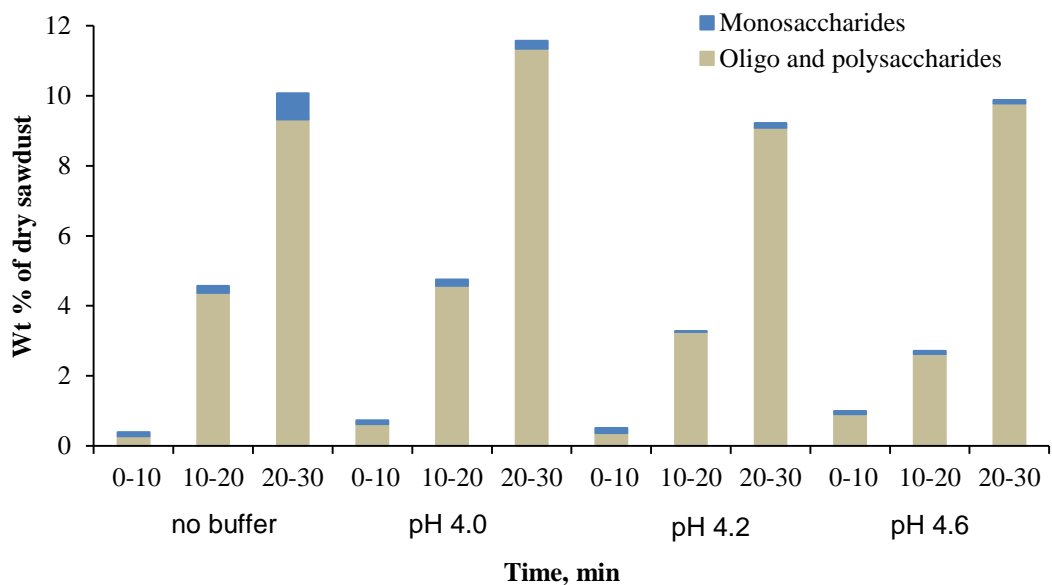


Fig. 7. The amount of extracted mono-, oligo-, and polysaccharides at 180 °C

A high carbohydrate yield was achieved with plain water at 170 °C. The extraction yields with pH buffers were significantly lower. Most of the monosaccharides were extracted with plain water, indicating higher hydrolytic cleavage of xylan. The yields of carbohydrates at 170 °C were higher than extractions at 160 °C. As with extraction at 160 °C, there were small amounts of glucose and fructose present in these extracts after the first 10 min of extraction.

The yields of oligo- and polysaccharides were similar between plain water and buffers at 180 °C. The yield with buffer of pH 4.0 was a little higher than with plain water. With buffers pH 4.2 and 4.6, yields were lower than for plain water and pH 4.0 buffer. This could be the result from the higher buffer pH values of 4.2 and 4.6 since yields at 10 to 20 min were lower than with plain water and pH 4.0 buffer. The pH 4.0 buffer seems applicable to inhibit hydrolysis at higher temperatures because it did not result in a decreased yield of extraction. There was a higher yield of monosaccharides at 20 to 30 min extraction with plain water than with buffers, indicating more hydrolytic cleavage of xylan. More xylan was released between 10 and 20 min extraction at 180 °C than at 170 °C. As a result, carbohydrate yields were higher at 180 °C than 170 °C.

Molar Masses of Xylan

The addition of sodium acetate buffer influenced the molar mass of the extracted non-cellulosic carbohydrates (Fig. 8). When plain water was used and the extraction temperature was increased from 160 °C to 180 °C, the average molar mass (M_w) of carbohydrates extracted between 20 to 30 min decreased from *ca.* 19 kDa to *ca.* 1 kDa. The addition of buffer, however, prevented hydrolytic cleavage of the extracted polysaccharides. Even after extraction at 180 °C, their average M_w was *ca.* 4 kDa. Indeed, on the SEC chromatogram of the extracts obtained with only water at 180 °C, the most pronounced peak was related to low molar mass hydrolysis products. After extraction under the same conditions using buffer, formation of these low molar mass compounds was suppressed. The M_w of extracts obtained at 160 °C with buffer addition was lower than that without buffer, due to a lower starting pH than plain water, *i.e.*, more substantial hydrolytic cleavage of the polysaccharide backbone was observed with lower pH values.

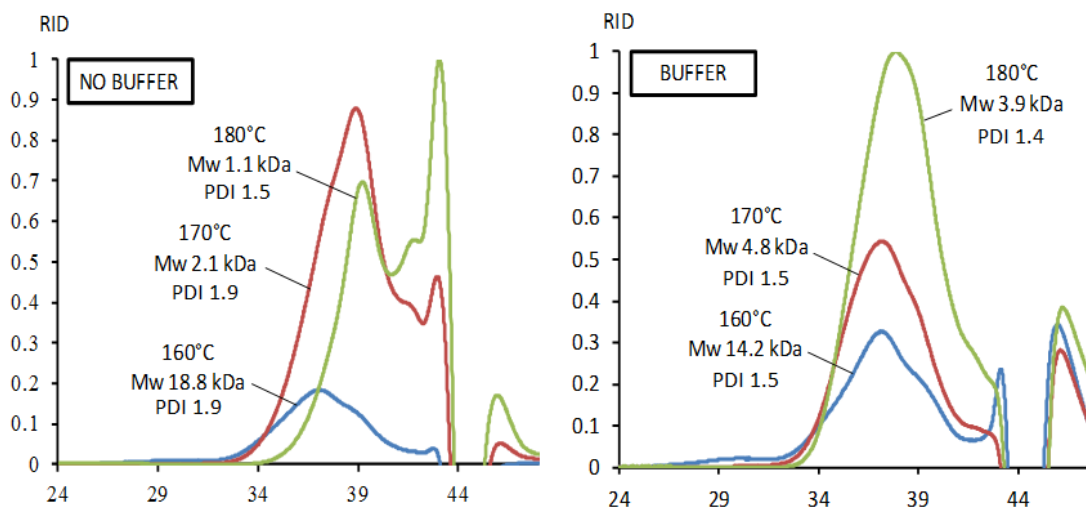


Fig. 8. HPSEC-MALLS chromatograms of extracts from birch wood, collected during the 20-30 min extraction time at different temperatures with and without the addition of pH 4.0 buffer

Severity of the Extractions

Five different severity factors were calculated from the extraction results. Temperatures inside the extraction vessels during extractions were measured and data from 160 °C, 170 °C, and 180 °C were used in calculations. Severity factors were calculated using Eqs. 2, 3, 4, and 5. Because the R_0 and P-factor calculations used temperature and pressure, the buffered extractions had the same R_0 and P-values in both plain water and buffered extractions. With the combined severity factor, $\log(R''_0)$ and modified P-factor pH values had to be taken into account in the calculations according to Pedersen and Meyer (2010).

To calculate the severity factors, third degree polynomials were fitted using the least squares method. To calculate the total severity of the extraction with a combined severity factor using pH calculations, a logarithmic curve was fitted for plain water extraction to calculate the pH during extraction and linear fits were used for buffered extraction calculations. Calculated severity factors are listed in Table 3.

Table 3. Severity Factor (R_0), P-factor, $\log(R_0)$, Combined Severity Factor ($\log(R''_0)$), and Modified P-Factor.

	Temperature, °C	R_0 , min	P-factor, h	$\log(R_0)$	$\log(R''_0)$	Mod P
PHWE	160	960	70	3.0	6.4	5.2
	170	1800	150	3.3	7.5	6.4
	180	3400	310	3.5	8.1	7.0
pH 4.0	160	960	70	3.0	6.5	5.4
	170	1800	150	3.3	7.0	5.9
	180	3400	310	3.5	7.5	6.4
pH 4.2	160	960	70	3.0	5.7	4.8
	170	1800	150	3.3	5.9	5.3
	180	3400	310	3.5	6.2	5.9
pH 4.6	160	960	70	3.0	5.2	4.7
	170	1800	150	3.3	5.6	5.0
	180	3400	310	3.5	5.7	5.9

Extraction severity was calculated for the whole 30 min of extraction. The combined severity factors of plain water and pH buffers were different in the beginning of the extraction, at 0 to 10 min. In the beginning of the extraction, small amounts of easily hydrolyzed sugars were extracted from sawdust, which are not shown in the R_0 and P-factors. Still, R_0 and P-factors were calculated for fast-reacting xylan, but not for easily hydrolyzed sugars. R_0 and P-factor would be most useful for hardwood calculations. For other biomasses with different kinds of hemicellulose contents, proper activation energies should be calculated.

As with the combined severity factor, the modified P-factor yielded different values for different buffer extractions. The values of $\log R_0$ at different extraction temperatures in a flow-through system were in a similar range as in PHW batch extractions used to make composites (Hosseini *et al.* 2011a,b; Hosseini 2012; Paredes *et al.* 2009). Alkaline and neutral lignocellulosic pretreatments were in a similar $\log R_0$ range as a flow-through PHW extraction (Pedersen and Meyer 2010). The PHW extraction at 180 °C had a similar combined severity factor of 8.1 as a 10 min alkali treatment at 140 °C. A PHWE flow-through system has a constant flow through the

extraction vessel so the severity for the extract and for the extraction residue is different. Buffered extraction can modify the extraction severity as can be seen from the molar masses of hemicelluloses and severity factor calculations. A pH buffer could be used to protect sensitive extraction residues or extracts from hydrolysis. Buffered extractions also modify remaining extraction residue. Extraction residue could then have properties, which could not be achieved with a normal PHW extraction. Added acetate could have an effect on the solubility or stability of the extracted hemicellulose. Acetate affects the solubility and stability of proteins in the Hofmeister series.

In Figs. 9a and b, R_0 , P-factors, combined severity factors, and modified P-factors are plotted against extracted xylan (xylose and 4-O-methyl glucuronic acid) yield.

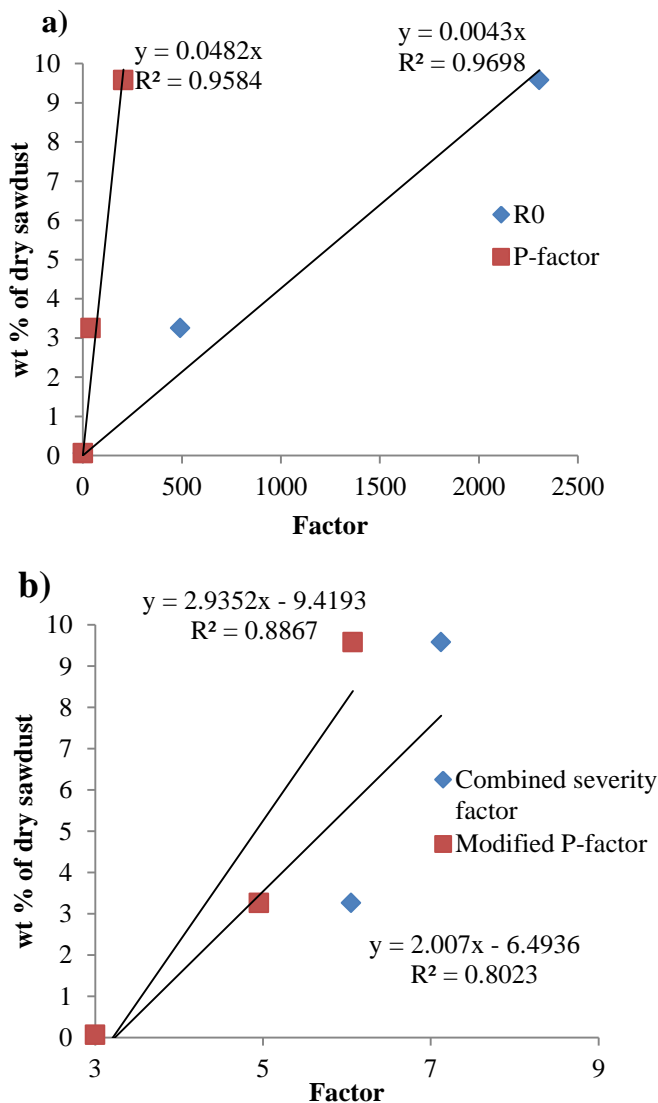


Fig. 9. (a) Severity factors R_0 and P-factor vs. xylan yield at 180 °C with pH 4.0 buffer; (b) combined severity factor and modified P-factor vs. xylan yield at 180 °C with pH 4.0 buffer

The R_0 and P-factor could be fitted linearly with extracted xylans. However, the combined severity factor and modified P-factor did not correlate as well linearly. At 160 °C and 170 °C with plain water and with pH buffer, R_0 and P-factor correlated better linearly with xylan yield than the combined severity factor or modified P-factor. On the

other hand, the change of pH with buffer was not taken into account, which had an effect on the xylan yield and the molar masses of extracted xylans at different extraction temperatures. The change of the pH during the extraction can have an effect on the activation energy of fast-reacting xylan hydrolysis (Abatzoglou *et al.* 1992).

The combined severity factor and modified P-factor gave different values for each pH buffer. Still, pH may have too large an effect on the combined severity factor and the modified p-factor in the beginning of the extraction since extraction temperature is still low. In order to create a more detailed model of different kinds of extractions, the change of pH during extraction should also be taken into account.

CONCLUSIONS

1. Pressurized hot water extraction using a flow-through system with an acetic acid/sodium acetate pH buffer is an efficient way to extract oligomeric and polymeric xylan from birch sawdust. The molar masses of the extracted carbohydrates were higher with buffers, and yields at 180 °C were comparable to PHW extraction.
2. Based on the results of this study, buffers could be used to reduce the hydrolysis of xylan. Buffer could be added at the point when the pH of the plain water extraction is at the buffer's initial pH.
3. The pH buffers have a more pronounced effect at higher extraction temperatures, inhibiting cleavage of xylan.
4. The severity factor, R_0 , and P-factor correlated better with xylan yield than the combined severity factor and modified P-factor.

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