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Enzyme-aided alkaline extraction of oligosaccharides and polymeric xylan from hardwood kraft pulp

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ABSTRACT

In this paper we describe the effect of enzyme treatments on the production of polymeric xylan, oligosaccharides and hemicellulose lean pulp by alkaline extraction of bleached hardwood kraft pulp. Enzyme treatments were carried out before one or in between two subsequent alkaline extractions by purified *Trichoderma reesei* xylanase and endoglucanase II (Cel 5a) as well as by a commercial monocomponent endoglucanase (FibreCareR). Without enzyme pre-treatment 61% and 7% of the pulp xylan was extracted in high purity in the first and second alkaline stage, respectively. Higher molecular mass xylan was obtained in the second than in the first alkaline extraction. Xylanase treatment before alkaline extraction hydrolyzed up to 12% of xylan to xylooligosaccharides. According to our results, preparation of polymeric xylan, and/or oligosaccharides as well as hemicellulose lean pulp with cellulose content of 93–94%, is possible by enzyme-aided alkaline extraction process.

1. Introduction

Currently the utilization of lignocellulose-based raw materials for novel end-uses is under vigorous investigation. The lignocellulose biorefinery research is greatly focusing on the production of biofuels and chemicals derived from abundant biomass resources. The production of several high value products such as polymeric hemicelluloses and oligosaccharides in addition to the main product could have a major impact on the economy of the biorefineries. The polymeric wood hemicelluloses, e.g. xylan and glucomannan, are interesting starting components for material applications, chemicals and liquid fuels. For example xylan as such or after modification has end-use applications in pulp and paper making, food and pharmaceutical industries (Ebringerová, Hromádková, & Heinze, 2005).

Potential value-added end-products obtained from kraft pulp, which is currently used mainly as paper grade pulp, are polymeric isolated hemicelluloses (Krogerus & Fuhrmann, 2009; Talja, Fuhrman, Krogerus, & Vähä-Nissi, 2009) and oligosaccharides (Rydlynd & Dahlman, 1997). Up to 60% of the xylan present in bleached hardwood market pulp can be isolated by alkaline extraction followed by precipitation and ultrafiltration (Talja et al., 2009). Xylan can also be isolated from the kraft cooking liquor (Dahlman, Tomani, Axegård, Lundqvist, & Lindgren, 2007) or from pulp prior to bleaching. Cold caustic extraction was used by Gomes, Colodette, Barbosa, and Oliveira (2011) to remove over 60% of xylan from unbleached eucalyptus kraft pulp. In comparison to sulphite pulps higher molecular weight xylans can be obtained from kraft pulp (Janzon, Saake, & Puls, 2008).

Xylooligosaccharides (XO) are already available especially on the Asian markets for use as food ingredients to stimulate the growth of beneficial bacteria in the intestinal tract (Vázquez, Alonso, Domínguez, & Parajo, 2000). XOs are produced from xylan rich feed-stocks from agriculture such as corn cobs and hulls but also wood based raw materials have been considered (Moure, Gullón, Domínguez, & Parajo, 2006). XOs can be manufactured by restricted acid hydrolysis, enzymatic hydrolysis, or hydrothermal treatment either directly or after fractionation of the feedstock (Vázquez et al., 2000). The advantage of enzymatic hydrolysis over acid hydrolysis is specificity and thus, although the process is slower, XOs with desired degree of polymerization (DP) are obtained without the formation of monosaccharides or furfural (Akipnar, Erdogan, Bakir, & Yilmaz, 2010).

With hydrolytic enzymes i.e. hemicellulases and cellulases different carbohydrate components from lignocellulosic raw materials and pulps can be selectively degraded. The selectivity of enzymatic treatment makes it an interesting process step when designing new process concepts. Several enzyme applications have been described for pulp and paper industry processes (Viikari, Suurnäkki, Grönqvist, Raaska, & Ragauskas, 2009). For example, up-grading hardwood kraft pulps into dissolving pulps has been successful by combining alkaline extraction and enzymatic

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Table 1
Enzyme activities and protein content of the used enzyme preparates.

	Xylanase (nkat/ml)	HEC[a] (nkat/ml)	Protein (mg/ml)
Trichoderma reesei, xylanase, pI 9	31 000	ND[b]	3.2
Trichoderma reesei, EG II (Cel5a)	11.5	6886	7.5
Humicola insolens, EG V	ND	1520	12.0

[a] HEC: hydroxyethylcellulose.
[b] ND: not detected.

treatment steps (Ibarra, Köpcke, & Ek, 2009; Köpcke, Ibarra, Larsson, & Ek, 2010). Paper grade birch kraft can be up-graded into dissolving grade pulps by two subsequent alkali extraction steps to decrease pulp xylan content followed by endoglucanase treatment to increase pulp reactivity (Köpcke et al., 2010). Similar results have been obtained with eucalyptus kraft pulp by xylanase-alkaline extraction-endoglucanase sequence (Ibarra et al., 2009). The utilization of the alkaline extract obtained from such process as a source of xylan to produce xylose has also been considered (Hyatt, Fengl, Edgar, & Alvarz-Wright, 1998).

Enzymatic treatments have separately been shown to have potential for the production of oligosaccharides from agricultural residues and for upgrading paper grade pulp into dissolving grade pulp. Based on the results by Talja et al. (2009), Ibarra et al. (2009) and Köpcke et al. (2010), the combination of alkaline extraction and enzymatic treatments causes formation of filtrates that contain 10–20% of the initial pulp carbohydrates. In order to make the process economically and environmentally feasible, these filtrates need to be exploited. However, so far the effect of the enzyme treatments on the DP of the carbohydrates present in these filtrates is not known. The possibility to obtain polymeric xylan and XOs as well as hemicellulose poor pulp from hardwood kraft pulp was evaluated in this study. The emphasis was on characterization of the effect of enzyme treatment on alkaline extraction and the effect on the molecular weight distribution of the extracted xylan as well as clarifying the possibility to isolate oligosaccharides from the pulp filtrates. In addition, the effect of enzyme treatments on the molecular weight distribution of kraft pulp polymers was clarified. Combination of xylanase or endoglucanase treatment and alkaline extraction of xylan were carried out and mass balance of the overall process was calculated.

2. Materials and methods

2.1. Materials

Enzyme treatments were carried out with xylanase (pI 9) purified from *Trichoderma reesei* culture filtrates as described by Tenkanen, Puls, and Poutanen (1992). Endoglucanase treatments were carried out with endoglucanase II (Cel5A) purified from *T. reesei* culture filtrate (Pere, Siika-aho, Buchert, & Viikari, 1995) (*Tr.* EG II) and with commercial endoglucanase product FibreCareR (Novozymes AS), which contains EG V from *Humicola insolens* (*Hi.* EG V). Xylanase and endoglucanase activities were determined as described by Pere et al. (1995) and the activities were expressed as katals. One nanokatal (nkat) of enzyme catalyzes the release of 1 nmol of reducing sugars from the substrate polymer (birch xylan for xylanase and hydroxyethylcellulose (HEC) for endoglucanase) in 1 s. Protein content of the enzyme preparates was determined with BIORAD protein assay. Xylanase and endoglucanase (HEC) activities and the protein content of the used enzyme preparates are presented in Table 1.

Bleached commercial hardwood kraft pulp (Södra Gold Birch Z) was utilized as raw material. Before enzyme treatments or alkaline extractions dry pulp was soaked overnight in water and disintegrated with Lorentz & Wettre pulp disintegrator in 60 g batches at 0.2% consistency for 30 000 revolutions.

2.2. Enzyme treatments

Enzyme treatments of pulp were carried out prior to one or in between two subsequent alkaline extraction steps. Pulp pH was adjusted to 5 with 0.5 M H_2SO_4. Xylanase treatment with xylanase dosage of 20 and 1000 nkat/g of dry pulp was carried out at 4% consistency, 45 °C and pH 5 for 2 h. Endoglucanase treatments with the enzyme dosage of 0.5 mg protein/g of dry pulp were carried out at 4% consistency, 45 °C and pH 5 for 2 or 24 h. Reference pulp was treated in the same way as described above for 2 h but without enzyme addition. During the enzyme treatments the pulp was mixed at 110–120 rpm. After the treatment, the pulp was heated to 90 °C for 15 min to inactivate the enzymes, and thereafter it was filtered with wire cloth and washed twice with 10 ml distilled water per g of pulp.

2.3. Alkaline extraction

Alkaline extraction of pulp was carried out as described by Talja et al. (2009) with 1 M NaOH at 5.5% consistency and at room temperature for 2 h with mixing at 70 rpm. After the alkaline extraction the pulp was filtered through wire cloth and washed thoroughly to remove the alkali. The alkaline extract contained the alkaline extract combined with the filtrate from the first washing step with 10 ml distilled water per g of pulp.

2.4. Determination of carbohydrate composition

To determine the carbohydrate composition of the pulps, pulp filtrates and extracted xylans the samples were hydrolyzed with sulphuric acid and analyzed according to Willför et al. (2009). Pulp samples were ground with Fritch pulverisette to pass 0.5 mm screen prior to acid hydrolysis. The resulting monosaccharides were determined by HPAEC with pulse amperometric detection (Dionex ICS 3000A) equipped with CarboPac PA1 column. The polysaccharide content in the samples was calculated from the corresponding monosaccharides using an anhydro correction of 0.88 for pentoses and 0.9 for hexoses. Linear oligosaccharides present in the pulp filtrates were analyzed by HPAEC with pulse amperometric detection (Dionex ICS 3000A) equipped with CarboPac PA1 column without the acid hydrolysis (Tenkanen, Makkonen, Perttula, Viikari, & Teleman, 1997). Linear XOs, xylobiose, xylotriose, xylotetraose, xylopentaose and xylohexaose (Megazyme) as well as cellobiose (Serva), cellotriose (Seikagaku), cellotetraose (Merck), cellopentaose (Seikagaku) and cellohexaose (Seikagaku) were used as standards. The mass balance of enzyme treatment and alkaline extraction was calculated as percentage of the original, un-extracted pulp.

2.5. Determination of molecular weight of extracted xylan and pulps

Molar mass measurements of xylan were performed by size-exclusion chromatography (SEC) in 0.1 M NaOH using PSS's MCX 1000 and 100 000 columns with a precolumn (0.5 ml/min, $T = 30$ °C). For pulp molar mass measurements, the extracted pulp samples were dissolved in DMAc/8% LiCl according to the solvent exchange method with ethylisocyanate derivatization (Berthold, Gustafsson, Sjöholm, & Lindstrom, 2001). The SEC measurements were performed using 2× PL gel MiniMixed A columns with a precolumn in DMAc/0.8% LiCl eluent (0.36 ml/min, $T = 80$ °C). In both cases, the

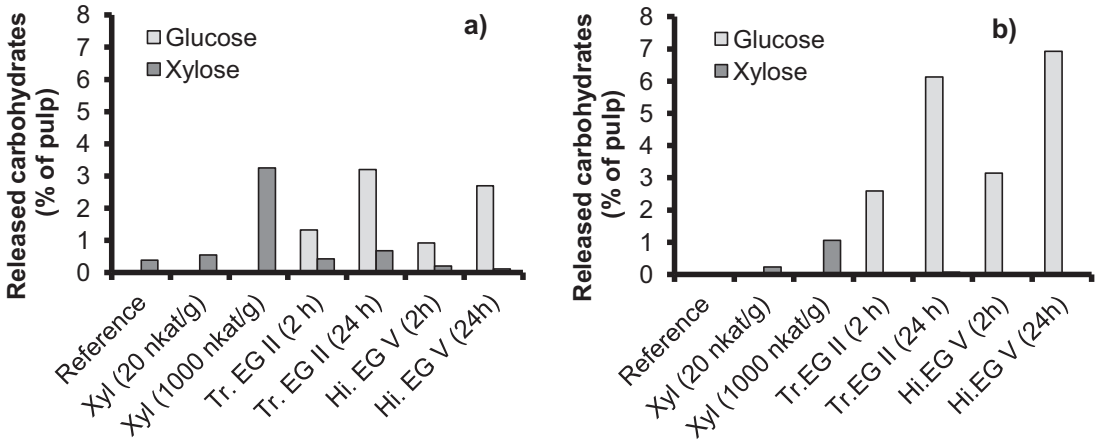

Fig. 1. Carbohydrates released from the un-extracted (a) and once alkali-extracted (b) bleached HW kraft pulp in the enzyme treatment. Analysis was carried out after acid hydrolysis by HPAEC and calculated as % of the dry pulp in the treatment. Reference: no enzyme addition; Xyl: *Trichoderma reesei* pI 9 xylanase, 2 h; Tr. EGII: *Trichoderma reesei* EG II (Cel 5A) 0.5 mg/g pulp; Hi. EGV: *Humicola insolens* EG V (FibreCareR) 0.5 mg/g pulp.

refractive index (RI) detector was used, and the molar mass distributions of xylan and pulp polysaccharides were calculated in relation to pullulan standards using Waters Empower 2 software.

3. Results and discussion

3.1. Enzyme hydrolysis efficiency in the original and alkali extracted pulp

Efficiency and specificity of the enzymatic hydrolysis of the bleached hardwood (HW) kraft pulp before and after alkaline extraction was quantified based on carbohydrates released from the pulp in the enzyme treatment. The analysis of the carbohydrates was carried out from the pulp filtrates after acid hydrolysis to monosaccharides. It was noted from the carbohydrates released to pulp filtrate that the treatments with xylanase and *H. insolens* EG V were quite specific towards xylan and cellulose, respectively (Fig. 1). *T. reesei* endoglucanase II released some xylose to the filtrate, which is probably caused by the minor xylanase impurity in the preparate (Table 1). Xylanase treatment (1000 nkat/g) hydrolyzed 2.9% of the un-extracted, original hardwood kraft pulp and only 1.1% of the pulp after the alkaline extraction (Fig. 1). The alkali-extracted pulp was hydrolyzed more efficiently than the un-extracted pulp by the both endoglucanases (Fig. 1).

It is well known that the enzyme reactions happen on the fibre surfaces and fines as the molecular size of the enzymes (5–10 nm diameter) hinders their penetration deep into the fibres (Suurnäkki et al., 1996). Xylan of the bleached HW kraft fibres is enriched at the fibre surface (Dahlman, Jacobs, & Sjöberg, 2003) and in fines (Lyytikäinen, Saukkonen, Kajanto, & Käyhkö, 2011). Thus, it can be speculated that xylan at the surface of un-extracted HW kraft pulp fibres is easily accessible to xylanase but hinders the hydrolysis by EGs. In addition, alkaline extraction increases the fibre porosity (Lyytikäinen et al., 2011), which enhances the penetration of the enzymes into the fibres and may have affected the hydrolysis efficiency of alkali-extracted pulp by xylanase.

The extensive removal of xylan and the subsequently increased fibre porosity by alkaline extraction apparently improved the accessibility of fibre cellulose to endoglucanases. Increased efficiency of endoglucanase hydrolysis after alkaline extraction has also been observed by Köpcke et al. (2010). In their study the enhanced hydrolysis was observed as increased reduction of pulp viscosity by commercial endoglucanase (Novozym 476) treatment of pulp after three alkaline extractions in comparison to pulp after two alkaline extractions. Lower molar mass of pulp polysaccharides, i.e. cellulose, was also detected in our experiments, when endoglucanase treatment was performed between two alkaline extractions (Fig. 3, Table 2).

3.2. Release of oligosaccharides to pulp filtrates

Oligosaccharides formed during the enzyme treatments of original and alkali-extracted bleached HW kraft pulp were analyzed by HPAEC-PAD from the pulp filtrates. As expected, xylooligosaccharides (XO) were formed by xylanase treatment and cello-oligosaccharides by EG treatments (Fig. 2). By comparing the amount of oligosaccharides (Fig. 2) and the total amount of released carbohydrates (analysis after acid hydrolysis, Fig. 1) it can be concluded that the carbohydrates were released to the filtrate mainly as oligosaccharides. XOs with DP two to six were released by the xylanase treatment, the main two hydrolysis products being xylobiose and xylotriose, which is in agreement with previous results (Tenkanen et al., 1992). The yield of XOs with the higher xylanase dosage (1000 nkat/g) was 12% or 3.8% of the original pulp xylan when the enzyme treatment was carried out to un-extracted or alkali-extracted pulp, respectively. The obtained DP profile of the pulp hydrolysate after xylanase treatment shows potential, as DP two to four XOs are preferred for food applications (Vázquez et al., 2000).

The cello-oligosaccharide profile released by the two endoglucanases differed from each other. *T. reesei* EGII released cellobiose and cellotriose as well as glucose, whereas *H. insolens* released longer oligosaccharides, mainly cellobiose, cellotriose and cellotetraose from the pulp.

3.3. Analysis of extracted xylans

Carbohydrate composition of alkaline extracts from enzyme aided xylan extraction was determined after pH adjustment and acid hydrolysis with HPAEC-PAD (Table 2). When endoglucanase treatment preceded the alkaline extraction also glucose was present in the alkaline extracts, whereas other xylan samples were of high purity. These results, carried out with bleached HW pulp comprising mainly of birch, are in accordance with the previously reported results showing that high purity (98–99% carbohydrates) xylan can be obtained from bleached birch pulp (Janzon et al., 2008; Talja et al., 2009).

The xylan content in alkaline extracts derived from the first extraction of the pulp was tenfold higher than that from the second extraction step (Table 2). Xylan yield of the reference pulp in the

Table 2
Composition, yield and average molar mass (Mn, Mw) of alkaline extracts from enzyme aided xylan extraction of HW kraft pulp.

Treatment sequence[a]	Content in alkaline extract (%)		Average molar masses			Xylan yield (%)[b]
	Xylan	Glucose	Mn	Mw	PD	
First extraction stage						
Reference–Alk	5.3	ND[c]	27 400	36 300	1.3	61
Xylanase 20 nkat–Alk	4.8	ND	25 200	34 900	1.4	55
Xylanase 1000 nkat–Alk	4.4	ND	16 600	24 700	1.5	50
Tr. EG II, 2 h–Alk	5.0	0.05	25 800	35 300	1.4	58
Tr. EG II, 24 h–Alk	4.9	0.05	23 900	32 900	1.4	56
Hi. EG V 2 h–Alk	5.7	0.07	26 700	36 700	1.4	64
Hi. EG V 24 h–Alk	5.1	0.07	26 800	36 600	1.4	58
Second extraction stage						
Alk–reference–Alk	0.6	ND	31 000	41 300	1.3	7.2
Alk–xylanase 20 nkat–Alk	0.7	ND	19 700	29 300	1.5	8.3
Alk–xylanase 1000 nkat–Alk	0.7	ND	12 700	20 000	1.6	8.0
Alk–*Tr.* EG II, 2 h–Alk	0.8	0.1	31 800	42 000	1.3	9.0
Alk–*Tr.* EG II, 24 h–Alk	0.8	0.1	26 500	35 700	1.4	9.6
Alk–*Hi.* EG V 2 h–Alk	0.6	0.2	31 700	41 200	1.3	7.3
Alk–*Hi.* EG V 24 h–Alk	0.7	0.2	30 900	40 100	1.3	8.2

[a] Reference: no enzyme addition; xylanase: *Trichoderma reesei* pI 9 xylanase, 2 h; *Tr.* EGII: *Trichoderma reesei* EG II (Cel 5A) 0.5 mg/g pulp; *Hi.* EGV: *Humicola insolens* EG V (FibreCareR) 0.5 mg/g pulp. Alk: alkaline extraction: 1 M NaOH at 5.5% consistency, room temperature, 2 h.
[b] Xylan yield as % of xylan present in original, un-extracted pulp.
[c] ND: not detected, detection limit 4 mg/l.

first extraction was 61% of the original pulp. Xylanase treatment decreased the extracted xylan yield by up to 10%, which corresponds to the yield of xylooligosaccharides (XOs) in the pulp filtrate (Table 2, Fig. 2). The extraction of xylan was not affected by the endoglucanase treatment, suggesting that the enzyme treatment has not improved the penetration of the alkali or altered the binding or xylan to cellulose.

After the second alkaline extraction of pulp, 7% of xylan present in the original un-extracted pulp was observed in the extract. The effects of the enzyme pre-treatments on the xylan yield in the second extraction, varying from 7 to 9% of original pulp xylan, were only minor.

Molar mass distributions of the isolated, alkali-extracted, xylans were determined by SEC in 0.1 M NaOH (Table 2). In the reference case the xylan extracted in the second stage had somewhat higher molar mass than that extracted in the first stage. Xylanase treatment decreased the molecular mass of the isolated xylan in both extraction stages, although the effect was more pronounced

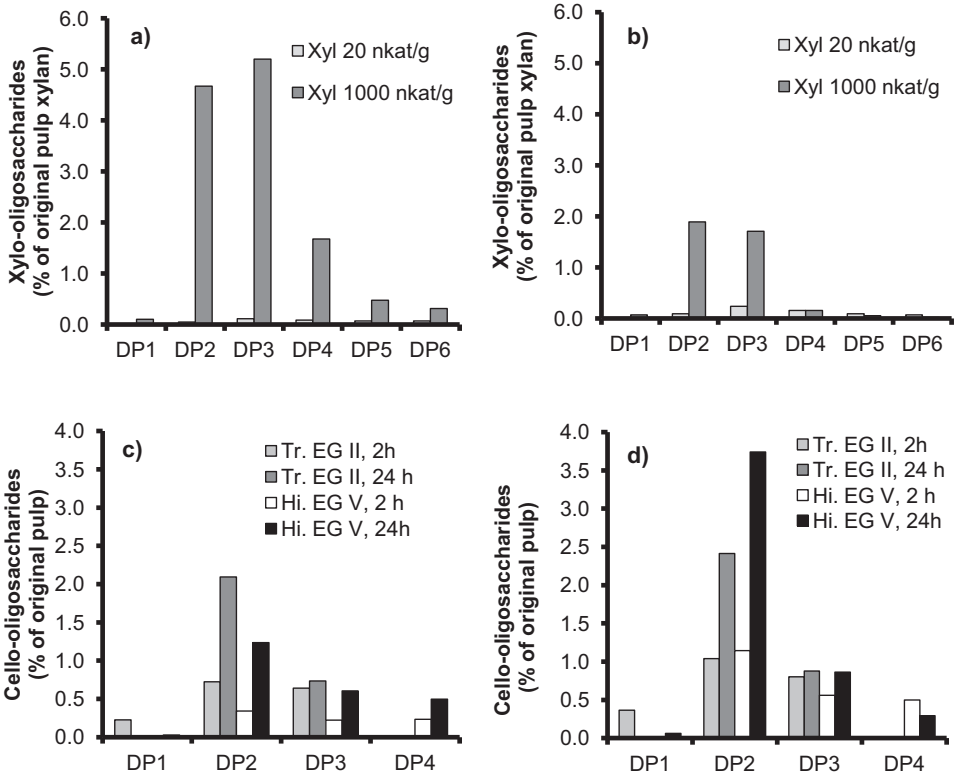

Fig. 2. Mono- and oligosaccharides formed during the enzyme treatment of un-extracted and alkali-extracted HW kraft pulp. Xylooligosaccharides (XO) released during xylanase treatment of un-extracted (a) and extracted (b) pulp and cello-oligosaccharides formed during EG treatments of un-extracted (c) and extracted (d) pulp. Reference: no enzyme addition; Xyl: *Trichoderma reesei* pI 9 xylanase, 2 h; Tr. EGII: *Trichoderma reesei* EG II (Cel 5A) 0.5 mg/g pulp; Hi. EGV: *Humicola insolens* EG V (FibreCareR) 0.5 mg/g pulp.

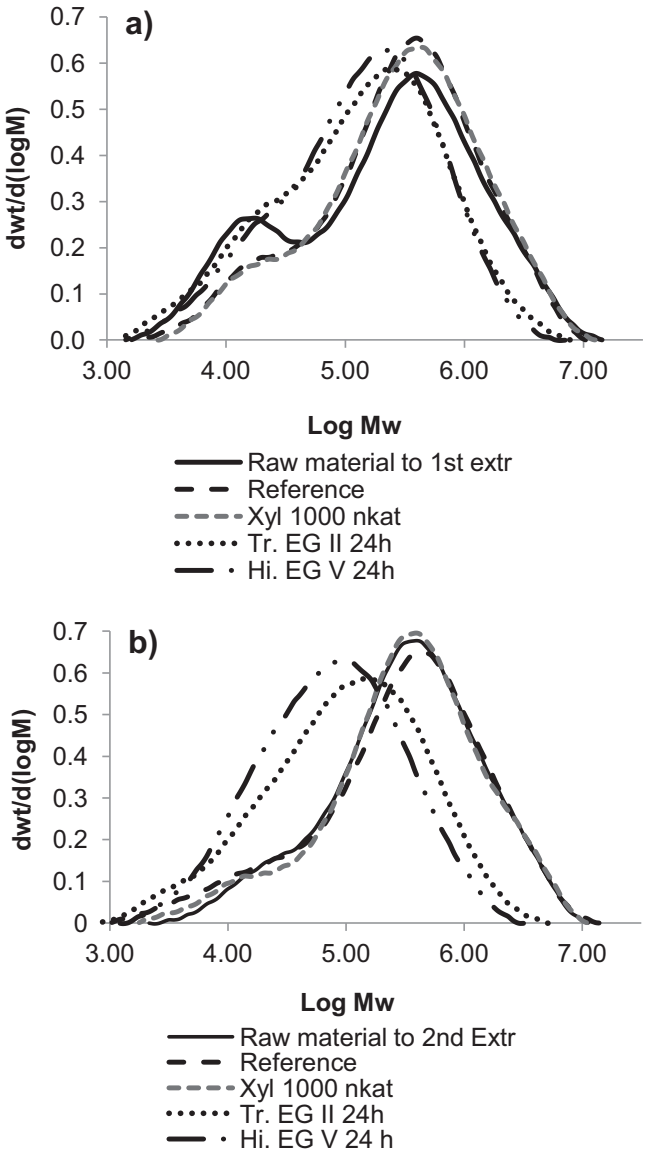

Fig. 3. Effect of enzyme treatments on molar mass distributions of the pulp polysaccharides after the first (a) and the second (d) alkaline extraction. Reference: no enzyme addition; Xyl: *Trichoderma reesei* pI 9 xylanase, 2 h, 1000 nkat; Tr. EG II: *Trichoderma reesei* EG II (Cel 5A) 0.5 mg/g pulp, 24 h; Hi. EG V: *Humicola insolens* EG V (FibreCareR) 0.5 mg/g pulp, 24 h.

in the second stage. This suggests that pulp xylan after first alkaline extraction was more efficiently hydrolyzed by the xylanase treatment in comparison to the un-extracted pulp.

The observed decrease in the molecular weight of xylan isolated from xylanase treated pulp was expected. A decrease in the molecular weight of xylan extracted from xylanase treated O-delignified eucalyptus kraft pulp was observed also by Gehmayr, Schild, and Sixta (2011). However, the used xylanase dosage was apparently higher than that used in our work as even 46% of pulp xylan was hydrolyzed and xylan Mw was 50% lower than that of the reference.

Endoglucanase treatment had smaller effect on xylan molar mass. It should be noted that the *T. reesei* EG II preparate contained also minor xylanase activity (Table 1), probably causing the observed decrease in xylan Mw observed especially in the second extraction after 24 h treatment. *H. insolens* EG V was specific towards cellulose and did not decrease the molar mass of extracted xylan even after long incubation time. An additional low molecular weight (Mw ~3500 Da) fraction was observed in the extracts derived from EG V treated pulps (results not shown). This fraction probably is derived from cellulose degradation products from enzymatic hydrolysis.

The molar mass distribution obtained in our work is comparable to that obtained by Talja et al. (2009). However, considerably lower molar mass xylan, Mw 11 000 Da was obtained by Janzon et al. (2008) from birch kraft pulp by using KOH, NaOH and nitren extractions. This supports the suggestion by Janzon et al. (2008), that DP of xylans extracted from pulps depend on the pulping conditions and thus differences in Mw of xylans isolated from different pulps are expected. It should also be noted, that the molar mass detection method used here is relative, and very much dependent on used measuring conditions, e.g. used eluent.

3.4. Properties of alkali-extracted pulps

Composition of kraft pulp samples from the sequential alkaline extraction and enzyme treatments are presented in Table 3. Pulp xylan content was 10–12% after the first and 6–7% after the second alkali-extraction stage. The decrease in xylan content in xylanase treated samples is comparable to the amount of xylan hydrolyzed during the enzyme treatment prior to the first alkaline extraction (Table 2, Fig. 1). After the second alkaline extraction only minor differences were observed in the composition of the reference and enzyme treated pulp samples.

Analysis of pulp molar mass distribution after different treatments indicated that the alkaline extraction of xylan prior to the endoglucanase treatment enhances the hydrolytic effect of endoglucanases on pulp cellulose (Fig. 3, Table 3). Based on the pulp molar mass distribution the cellulose hydrolysis was more efficient with *H. insolens* EG V compared to *T. reesei* EG II and the difference is even more emphasized when EG treatment is performed after alkaline extraction (Fig. 3). In addition the pulp molar mass distribution was narrower after the treatment with *H. insolens* EG V than *T. reesei* EG II. The higher reduction of cellulose molar mass by endoglucanases between alkaline extractions is well in accordance with the results of Köpcke et al. (2010). The xylanase treatments had no effect on molar mass distribution of pulp polysaccharides analyzed after alkaline extractions.

3.5. Mass balance of enzyme-aided xylan extraction

The mass balance of the enzyme aided alkaline extraction of birch kraft pulp with one or two subsequent alkaline extraction steps are presented in Table 4. The total yield comprises of the extracted pulp, oligosaccharides and other carbohydrates analyzed from the pulp filtrate, and xylan and other carbohydrates analyzed from the alkaline extract. When the enzyme treatment was carried out between two subsequent alkaline extraction steps the mass balance was calculated as percentage from the original unextracted pulp, making the theoretical overall yield 85%. The total yield of the first alkaline extraction step varied from 90 to 101%. The low 90% overall yields were observed for pulps with the most extreme enzyme treatment, either high xylanase dosage or long incubation time with endoglucanase. The possible explanation for the low overall yield observed in these cases is that some of the pulp material has been lost during pulp washing steps. In the treatment of alkali-extracted pulp the total yields are close to 100% of the theoretical maximum.

The xylan yield of the reference sample in the first alkaline extraction stage was 15% of the original pulp and 61% the original pulp xylan. The xylan yield was decreased by the extensive xylanase treatment to 12% of the original pulp, with simultaneous 2.8% yield of xylooligosaccharides (XO) in the pulp filtrate (Table 4). In the two stage alkali-extraction, the extracted xylan yield after the second extraction step was about 2% of the original pulp 7–9% of the xylan

Table 3
Composition, average molar mass and yield of alkali-extracted pulps with or without enzyme treatment.

Treatment sequence[a]	Xylan (%)	Cellulose (%)	Other carboh. (%)	Pulp yield (%)[b]	Average molar masses		
					Mn (g/mol)	Mw (g/mol)	PD
Raw material to 1st extraction stage experiments	23.6	75.9	0.5	100	42 300	728 900	17.2
Reference–Alk	12.0	87.5	0.5	83	67 900	721 000	10.6
Xylanase 20 nkat–Alk	11.3	88.3	0.5	82	64 800	725 600	11.2
Xylanase 1000 nkat–Alk	10.6	88.9	0.5	75	77 000	775 000	10.1
Tr. EG II, 2 h–Alk	12.4	87.1	0.5	78	46 400	469 700	10.1
Tr. EG II, 24 h–Alk	12.7	86.8	0.5	80	34 800	377 200	10.8
Hi. EG V 2 h–Alk	10.2	89.4	0.4	84	47 100	397 100	8.4
Hi. EG V 24 h–Alk	10.6	89.0	0.5	73	41 800	339 700	8.1
Raw material to 2nd extraction stage experiments	9.3	90.2	0.5	85	95 500	782 600	8.1
Alk–reference–Alk	6.2	93.5	0.4	83	52 600	801 500	15.2
Alk–xylanase 20 nkat–Alk	7.1	92.4	0.5	83	61 200	760 600	12.4
Alk–xylanase 1000 nkat–Alk	6.4	93.1	0.5	82	77 000	790 600	10.2
Alk–*Tr.* EG II, 2 h–Alk	7.1	92.4	0.5	81	41 900	365 400	8.7
Alk–*Tr.* EG II, 24 h–Alk	5.6	94.0	0.4	78	27 600	262 700	9.5
Alk–*Hi.* EG V 2 h–Alk	7.0	92.6	0.5	80	26 800	221 100	8.2
Alk–*Hi.* EG V 24 h–Alk	6.7	92.8	0.4	77	30 200	180 300	6.0

[a] Raw material for the 1st extraction stage is un-extracted commercial HW bleached kraft pulp, raw material for the 2nd extraction stage is the same pulp after one alkaline extraction stage. Reference: no enzyme addition; xylanase: *Trichoderma reesei* pI 9 xylanase, 2 h; *Tr.* EGII: *Trichoderma reesei* EG II (Cel 5A) 0.5 mg/g pulp; *Hi.* EGV: *Humicola insolens* EG V (FibreCareR) 0.5 mg/g pulp; Alk: alkaline extraction with 1 M NaOH at 5.5% consistency, room temperature, 2 h.
[b] Pulp yield as % of the original, un-extracted pulp.

Table 4
Mass balance of enzyme-aided first and second alkaline extraction stage of HW kraft pulp as percentage of the original pulp.

Treatment sequence[a]	Pulp yield (%)	Oligosac. yield (%)[b]	Other carboh. in filtrate (%)	Xylan yield (%)	Other carboh. in xylan (%)	Total yield (%)[c]
Reference–Alk	83	ND[d]	0.4	15	ND	98
Xylanase 20 nkat–Alk	82	0.1	0.5	13	ND	96
Xylanase 1000 nkat–Alk	75	2.8	0.4	12	ND	90
Tr. EG II, 2 h–Alk	78	1.4	0.4	14	0.13	94
Tr. EG II, 24 h–Alk	80	2.8	1.1	14	0.13	98
Hi. EG V 2 h–Alk	84	0.8	0.3	16	0.19	101
Hi. EG V 24 h–Alk	73	2.3	0.5	14	0.20	90
Alk–reference–Alk	83	ND	ND	1.5	ND	85
Alk–xylanase 20 nkat–Alk	83	0.1	0.1	1.7	ND	85
Alk–xylanase 1000 nkat–Alk	82	0.8	0.1	1.7	ND	84
Alk–*Tr.* EG II, 2 h–Alk	81	1.8	0.3	1.9	0.2	86
Alk–*Tr.* EG II, 24 h–Alk	78	3.3	1.9	2.0	0.3	86
Alk–*Hi.* EG V 2 h–Alk	80	2.2	0.5	1.5	0.4	85
Alk–*Hi.* EG V 24 h–Alk	77	4.9	1.0	1.7	0.5	85

[a] Reference: no enzyme addition; xylanase: *Trichoderma reesei* pI 9 xylanase, 2 h; *Tr.* EGII: *Trichoderma reesei* EG II (Cel 5A) 0.5 mg/g pulp; *Hi.* EGV: *Humicola insolens* EG V (FibreCareR) 0.5 mg/g pulp. Alk: alkaline extraction with 1 M NaOH at 5.5% consistency, room temperature, 2 h.
[b] Xylooligosaccharides in the case of xylanase and cellooligosaccharides in the case of EG.
[c] In the second extraction stage the theoretical maximal yield is 84% of original un-extracted pulp.
[d] ND, not detected, detection limit 4 mg/l.

present in the original un-extracted pulp depending on the enzyme treatment used (Tables 3 and 4).

4. Conclusions

Hemicellulose poor pulps as well as oligosaccharides and polymeric xylan were obtained by combining specific enzymatic treatments and alkaline extraction. Sequential alkaline extraction of hardwood kraft pulp yielded two high purity xylan fractions, one with high yield (60% of original pulp xylan) and one with high molecular weight (up to 40 000 Da) but considerably lower yield (~7% of original pulp xylan). Reduction of cellulose DP by both *T. reesei* EG II and *H. insolens* EG V was significantly enhanced after xylan removal.

Xylanase treatment prior to alkaline extraction decreased the yield and molecular weight of extracted xylan but provided the possibility to isolate xylooligosaccharides (~10% of original xylan) as an additional value added component. To obtain xylan with un-altered molecular weight as well as xylooligosaccharides (3.8% of original xylan), xylanase treatment should be carried out after alkaline extraction of xylan. Thus, depending on the desired xylan fraction, the xylanase treatment should be omitted (polymeric xylan with high DP obtained), carried out before the alkaline extraction (xylooligosaccharides and polymeric xylan with decreased DP obtained) or after the alkaline extraction (polymeric xylan with high DP obtained and xylooligosaccharides obtained).

The effects of EG treatments on the pentose containing filtrates were minor and xylan extracts comparable to that from reference were obtained after EG treatments. Thus EG treatment does not negatively affect the extraction of polymeric xylan. The main benefits obtained by EG treatment lies in its positive effect on the pulp reactivity (Köpcke et al., 2010) and DP. In our experiments *H. insolens* EG V decreased the pulp polydispersity, which is a desired property for a dissolving grade pulp.

It is thus possible to utilize similar process sequence than previously used for upgrading hardwood kraft pulp into dissolving pulp for obtaining both polymeric xylan and XOs together with hemicellulose lean hardwood kraft pulp.

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