Carbohydrate Polymers 79 (2010) 17–25

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Extraction and characterization of hemicelluloses from flax shives by different methods

Anvar U. Buranov, G. Mazza *

*Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Summerland, BC, Canada V0H 1Z0*

ARTICLE INFO

*Article history:*
Received 27 April 2009
Accepted 19 June 2009
Available online 25 June 2009

*Keywords:*
Flax
*Linum usitatissimum*
Polysaccharides
Hemicellulose
Xylooligomers
Extraction
Purification
Pressurized water
Aqueous ethanol
Microwave-assisted
Partition chromatography
Size exclusion chromatography
Spectroscopy
FT-IR

ABSTRACT

Hemicelluloses were extracted from flax shives using pressurized low-polarity water (PLPW), pressurized aqueous ethanol (PAE), microwave-assisted water (MW-Water) or aqueous ethanol (MW-EtOH), and precipitated with ethanol. Hemicelluloses still remaining in solution were further separated using ultrafiltration. All samples were characterized with chemical analysis, ion-moderated partition chromatography (IMP), size exclusion chromatography (SEC), and Fourier transform infrared (FT-IR) spectroscopy. PLPW, PAE, MW-Water and MW-EtOH extracted 90, 80, 18, and 40% of the total hemicelluloses, respectively. The molecular weight of the ethanol-precipitated hemicelluloses ranged from approximately 11,000 to 40,000 Da and the ethanol-soluble low-molecular weight hemicelluloses were about 1700 Da. High-molecular weight hemicellulose isolated from PAE extracts contained ~23% lignin, while that from the PLPW extracts contained ~5% lignin. Low-molecular weight hemicelluloses separated by ultrafiltration from PLPW and PAE extracts contained similar amounts of lignin (~20%). However, the yield of low-molecular weight hemicelluloses from PLPW was higher (~15%) compared to that from PAE (~6%). The FT-IR results revealed the specific band maximum at 1220 $cm^{-1}$ and the bands between 1175 and 1000 $cm^{-1}$ which are typical of xylans.



## 1. Introduction

Flax (*Linum usitatissimum* L.) is grown worldwide, mostly for its fiber and seed oil. Flax shives are the woody residue of straw left after the removal of fibers by retting and scutching. The yield of shives is 2.5 tonnes per tonne of fiber produced (Cox, El-Shafey, Pichugin, & Appleton, 1999). Fractionation of flax shives into constituent biopolymers and their applications represent a significant economic opportunity due to the large quantity of flax shives available worldwide (Buranov & Mazza, 2008).

Previously, we have reported fractionation of flax shives using environmentally friendly processes such as pressurized low-polarity water (PLPW), pressurized aqueous ammonia (PAA), and pressurized aqueous ethanol (PAE). The hemicelluloses obtained were initially characterized with Fourier transform infrared (FT-IR) spectroscopy (Buranov & Mazza, 2007; Buranov & Mazza, 2009). Extraction of hemicelluloses with liquid hot water is generally explained by the catalysis of acetyl groups liberated during the breakdown of bonds in biomass and the hydronium ions of water; however, recently reported results with acetic acid did not confirm the involvement of the acetyl group (Liu & Wyman, 2003). The presence of a small amount of acid (~1% $H_2SO_4$) increased the hemicellulose removal from 65% to 90%, confirming the role of hydronium ions in the water (Grohmann, Torget, & Himmel, 1985; Torget, Walter, Himmel, & Grohmann, 1991). However, the use of acids is undesirable due to corrosion of extraction vessels, the degradation of hemicellulose into monomers, and the formation of furfural (Teramoto, Tanaka, Lee, & Endo, 2008). Alkaline extractions of hemicelluloses are used for lower temperature and pressure extractions. Alkaline solutions hydrolyze the ester linkages to liberate hemicelluloses into aqueous media. Combination of alkaline extractions with hydrogen peroxide leads to the quantitative removal of both hemicellulose and lignin (Sun, Sun, Sun, & Su, 2004); however, environmental concerns and product recovery costs make the practical use of alkaline extractions challenging.

Microwave-assisted water extraction has been reported to be an efficient method for hemicellulose extraction in the shortest period of time (Jacobs, Palm, Zacchi, & Dahlman, 2003; Lundqvist et al., 2002). Hemicelluloses extracted from flax shives using a hydrothermal microwave treatment were characterized with respect to molar mass, molar mass distribution and degree of polymerization by employing size exclusion chromatography

* Corresponding author. Tel.: +1 250 494 6376; fax: +1 250 494 0755.
*E-mail address:* giuseppe.mazzag@agr.gc.ca (G. Mazza).

0144-8617/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2009.06.014

(SEC) in combination with matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (Jacobs et al., 2003). Microwave-assisted fractionation of NaOH-impregnated spruce into hemicelluloses has also been carried out (Lundqvist et al., 2002).

In recent years, there has been growing interest in the characterization of hemicelluloses extracted from different crop residues, and with different techniques, in order to find novel applications. Hemicelluloses have been used for the synthesis of cationic polymers (Ebringerová, Hromádková, Kacuráková, & Antal, 1994), hydrogels (Gabrielii, Gatenholm, Glasser, Jain, & Kenne, 2000), ester derivatives (Fang, Sun, Fowler, Tomkinson, & Hill, 1999), and thermoplastic derivatives (Jain, Sjöstedt, & Glasser, 2000). Hemicelluloses can also be used for the production of xylitol, a sweetener (Preziosi-Belloy, Nolleau, & Navarro, 1997) and furfural (Karimi, Kheradmandinia, & Taherzadeh, 2006). Hemicelluloses from wheat straw, sugarcane bagasse, and rice straw have been studied after alkaline extraction (Lawther, Sun, & Banks, 1995; Sun, Lawther, & Banks, 1996; Sun et al., 2004); however, hemicellulose extraction using environmentally friendly and benign extraction processes has received little attention.

Characterization of the hemicelluloses of flax shives is mainly focused on the alkaline extraction and determination of molecular properties of certain compounds such as 4-O-methylglucuronoxylan (Geerdes & Smith, 1955), O-acetylated xylan (Van Hazendonk, Reinerink, Waard, & van Dam, 1996) and xyloglucans (McDougall, 1993). No work has been done on the chemical characterization of hemicelluloses from flax shives extracted using different processes. The purpose of this work, therefore, was to characterize hemicelluloses that were isolated using environmentally friendly methods employing water and aqueous ethanol without acidic or alkaline additives and also to study the effect of conventional and microwave heating on process efficiency and on the quality of the hemicelluloses being isolated. In addition, the hemicelluloses were analyzed for monomeric sugars, xylooligomers, and molecular weights.

## 2. Materials and methods

### 2.1. Materials

Flax shives provided by Biolin Research Inc. (Saskatoon, Canada) were stored in a freezer at −35 °C until used. They were ground to a particle size of ~0.5 mm with a rotor mill (Retsch Company, Germany) and contained 32.5 ± 0.2% glucan, 16.5 ± 0.5% xylan, 23.7 ± 0.3% total lignin, and 3.3 ± 0.1% ash as determined by the standard methods published by the National Renewable Energy Laboratory (Sluiter et al., 2007).

### 2.2. Extraction and analysis

#### 2.2.1. Extraction of hemicellulose from flax shives in a PLPW extractor

Extraction with pressurized solvents was carried out in a pressurized low-polarity water (PLPW) extractor as described in our previous publications (Buranov & Mazza, 2007; Mazza & Cacace, 2005). Milled flax shives (13 g) were packed in a stainless steel extraction column (20 cm long × 2 cm ID) with frits at both ends. Extraction was started by pumping the extracting solvents (water or 30% ethanol) directly into the PLPW extraction system to bring the pressure up to 5.2 MPa. All experiments were carried out by passing the extracting solvent at 180 °C, flow rate of 3 mL/min, and solvent/feed 27 mL/g for 117 min determined as optimal for pressurized aqueous ethanol extraction (Buranov & Mazza, 2009). After extraction, the column containing solid residue was washed by pumping deionized water at room temperature. Solid residue was removed from the column and dried in a convection oven at 45 °C.

#### 2.2.2. Microwave-assisted extraction of hemicellulose from flax shives

Dry flax shives (13 g) were placed in 13 extraction vessel tubes (1 g in each tube) and 351 mL of water or 30% aqueous ethanol was added (27 mL to each tube). A magnetic stirrer was also added to improve mixing. The tubes were irradiated for the desired time (5, 10, and 20 min) at 180 °C in an ETHOS EX microwave (Milestone, Italy) with constant rotation for better irradiation and stirring. The liquid extracts were pooled together and filtered (Whatman #4) to separate them from the remaining flax shive residues. To the filtrate was added four volumes of 95% ethanol to precipitate the high-molecular weight hemicellulose (HMH). The liquid phase was separated from the precipitated HMH by settling at room temperature and decantation. Hemicellulose was further separated from the remaining liquid by centrifugation at 18,368*g* for 20 min at room temperature.

#### 2.2.3. Analysis of liquid extracts for free sugars

The liquid extracts (1.5 mL) were centrifuged at 12,100*g* for 5 min and filtered with a 0.45 μm nylon syringe filter. The samples were analyzed for simple sugars using an HPLC (Agilent model 1100) system equipped with a Bio-Rad Aminex HPX-87P column, a refractive index detector, and a G1329A autosampler using Agilent Chemstation Plus Software (Agilent Technologies, Palo Alto, CA).

#### 2.2.4. Separation of high-molecular weight hemicelluloses, lignin and ethanol-soluble low-molecular weight hemicelluloses in the extracts

To precipitate high-molecular weight hemicelluloses (HMH), four volumes of 95% ethanol were added to the liquid extracts (Bobleter, 1994). The liquid phase was separated from the precipitated HMH by settling at room temperature and decantation. HMH was further separated from the remaining liquid by centrifugation at 18,368*g* for 20 min at room temperature. The precipitated HMH were dried at 60 °C and weighed.

The supernatant was vacuum evaporated to remove ethanol and the lignin was precipitated and separated from the remaining liquid by centrifugation at 18,368*g* for 20 min at room temperature. The precipitated lignin was dried at 60 °C and weighed. Analysis of the aqueous supernatant with IMP chromatography indicated that it still contained a significant quantity of low-molecular hemicelluloses (LMH) that were not separable with the EtOH precipitation due to their solubility in ethanol; therefore, supernatant was placed in an ultrafiltration unit under nitrogen pressure at 344.7 Pa (AMICON 8400, Millipore Corp., MA, USA). TF (Thin Film) UF GH membrane with a cutoff of 1000 Da (Sterlitech Corp., Kent, Washington) was used. The term low-molecular weight hemicelluloses (LMH) or EtOH-soluble hemicellulose will be used to define hemicelluloses separated via ultrafiltration. LMH were dried at 45 °C.

#### 2.2.5. Analysis of solid residues and isolated hemicelluloses

Solid samples left after PLPW, PAE and microwave-assisted extractions, along with the HMH and LMH, were analyzed for glucan and xylan and lignin according to the NREL Chemical Analysis and Testing Standard Procedures (Sluiter et al., 2007). The samples were treated with 72% $H_2SO_4$ for 1 h at 30 °C in a water bath, diluted to 4% and autoclaved at 121 °C for 1 h. The hydrolysis solution was vacuum filtered on a filtering crucible (Coors #60531) and the content was measured gravimetrically by ashing in a muffle furnace at 575 °C. The filtrate was neutralized with $CaCO_3$ and filtered through 0.2 μm syringe filters. Sugar monomers were determined quantitatively with an HPLC system as described above.

*2.3. Ion-moderated partition chromatography (IMP) of xylooligomers*

*2.3.1. Determination of xylooligomers in the liquid extracts by IMP chromatography*

The liquid extracts from the pressurized and microwave-assisted extractions were further analyzed for xylooligomer content. The extracts were mixed thoroughly and a 1.5 mL sample was taken. The sample was centrifuged at 25,654*g* for 5 min at room temperature and filtered through a 0.45 μm nylon syringe filter. The transparent samples were analyzed by HPLC equipped with a refractive index detector (Model 1100, Agilent Technologies, Palo Alto, CA) and a Bio-Rad Aminex HPX-42A ion-moderated partition (IMP) column. The mobile phase was deionized water at a flow rate of 0.5 mL/min and the column temperature was 85 °C. Calibration curves of xylose, xylobiose, xylotriose, xylotetraose, and xylopentaose (Megazyme International Ireland Ltd., UK) were used for qualitative and quantitative determination of xylooligomers (Li, Converse, & Wyman, 2003).

*2.3.2. Determination of xylooligomers in the liquid extracts after precipitation of high-molecular weight hemicelluloses*

High-molecular weight hemicelluloses were precipitated from the liquid extracts by adding 4× vol. of 95% ethanol. The precipitated hemicelluloses were allowed to settle overnight and then the transparent liquid phase was decanted. Precipitated hemicellulose was centrifuged to remove the remaining liquid phase. Liquid phases were pooled together and the ethanol was vacuum evaporated. The remaining aqueous solution was again analyzed for xylooligomer content with an HPLC system as described above.

*2.4. Determination of the molecular weight distribution of hemicelluloses by size exclusion chromatography (SEC)*

Ethanol-precipitated high-molecular weight hemicelluloses were dissolved in 0.1 M $NaNO_3$ solution to prepare 2 mg/mL solution of xylans. The determination of molecular weights was carried out by HPLC equipped with Ultrahydrogel 500, 1000, and 2000 (Waters Ltd., Canada) linked in series to each other and capable of determining molecular weights of 1–1000 kDa. The refractive index detector was used for monitoring the peaks. The eluent system was 0.1 M $NaNO_3$ at a flow rate of 0.5 mL/min. The sample injection volume was 20 μL. The HPLC chromatogram from the refractive index detector was used for the comparison of retention times and contents. A dextran standard kit (Polymer Standard Service-USA, Warwick, RI) was used for comparison. Aqueous solutions (2 mg/mL) of dextran standards with molecular weights of 4400–750,000 were used to prepare the calibration curve. The signal from the RI detector was processed using PSS WinGPC Unity Software (Polymer Standard Service-USA, Warwick, RI).

*2.5. FT-IR spectra of hemicelluloses*

FT-IR spectra of isolated ethanol-precipitated hemicelluloses and soluble hemicelluloses were obtained with an FT-IR spectrometer "Nicolet-380" (Thermo Fisher Scientific Inc., Madison, WI, USA) using a Smart iTR® ATR sampling accessory (Sun et al., 2004).

## 3. Results and discussion

*3.1. Extraction and analysis*

The isolation protocol of hemicelluloses from flax shives is presented schematically in Fig. 1. HMH was precipitated from the extracts by adding 4× vol. 95% ethanol. The vacuum evaporation of ethanol resulted in the precipitation of lignin. LMH was separated from the remaining aqueous extract using ultrafiltration.

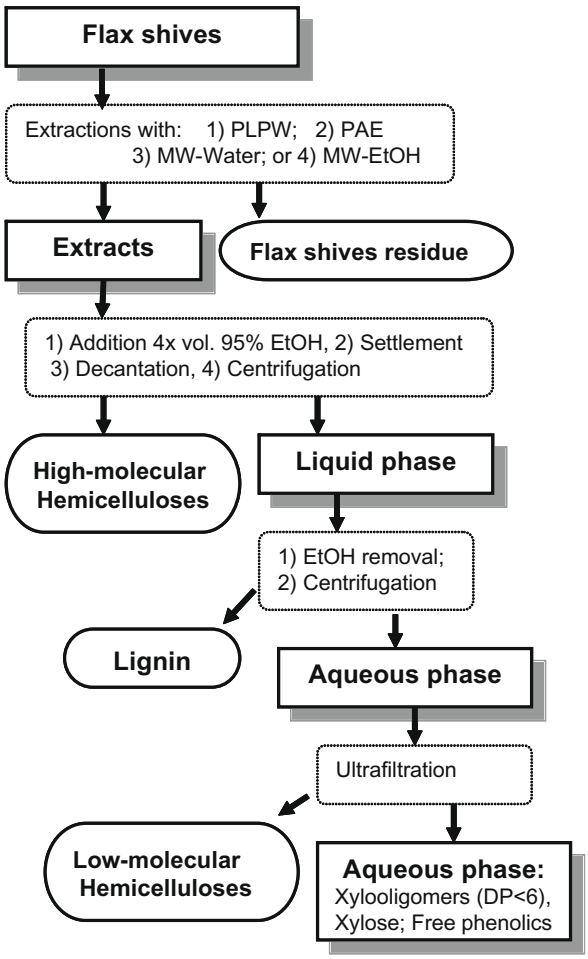


**Fig. 1.** Scheme for fractional isolation of hemicelluloses from flax shives using pressurized low-polarity water (PLPW), pressurized aqueous ethanol (PAE), Microwave-water (MW-Water), and Microwave-ethanol (MW-EtOH) extractions.

Experimental conditions and the HMH yields from flax shives using pressurized solvents and microwave irradiation with water and 30% ethanol are given in Table 1. Significant removal of biopolymers from flax shives was observed with PAE and the remaining solid residue was only 46.9%. Microwave-water treatment did not alter very much the amount of solid residue (83.5%). The maximum yield of HMH was observed with PAE (15% of dry flax shives) and the lowest yield was observed with microwave-water extraction (2%). Microwave-EtOH extraction was almost as efficient as PLPW and much more efficient than microwave-water extraction. Microwave heating for 20 min yielded less hemicellulose than heating for 10 min, probably due to degradation of hemicellulose; therefore, microwave heating for 10 min was chosen for further studies.

The liquid extracts before hemicellulose precipitation were also analyzed for free monosaccharides directly after filtering the extracts with a 0.45 μm nylon filter. Free sugars are usually derived from degradation of the polysaccharides during the extraction process (Liu & Wyman, 2003). Xylose was the main constituent sugar in the extracts from PLPW (34.6 mg/g) and PAE (14.4 mg/g), and mannose was the second major sugar (23.8 mg/g) (Table 2). Both microwave extractions (10 min) yielded less sugars (~1–5 mg/g) compared to PLPW and PAE. Arabinose content was the lowest in all liquid extracts (Table 2) and hemicellulose samples (Tables 4 and 6). The total amount of sugars was the highest (102.1 mg/g) with PLPW extractions, probably due to the increased degradation of sugars with water.

**Table 1**
Experimental conditions and high-molecular weight hemicellulose (HMH) yield from 13 g flax shives using PLPW, PAE, MW-Water and MW-EtOH at 180 °C, 5.2 MPa and a solvent/feed ratio of 27 mL/g.

| Exp # | Extraction method | Extraction mode: (solid/liquid ratio) | Flow rate, (mL/min) | Extraction time (min) | Solid residue (%) | Extract volume (mL) | HMH yield | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | (g) | (% on DFS) |
| 1 | PLPW | Flowthrough (1:27) | 3 | 117 | 54.2 | 351 | 0.81 ± 0.1 | 6.2 |
| 2 | PAE | Flowthrough (1:27) | 3 | 117 | 46.9 | 351 | 1.93 ± 0.3 | 15.0 |
| 3 | | Batch (1:27) | – | 5 | 84.3 | 351 | 0.20 ± 0.01 | 1.5 |
| 4 | *MW-Water | | | 10 | 83.5 | 351 | 0.27 ± 0.02 | 2.0 |
| 5 | | | | 20 | 83.8 | 351 | 0.23 ± 0.01 | 1.8 |
| 6 | | Batch (1:27) | – | 5 | 76.2 | 351 | 0.59 ± 0.03 | 4.5 |
| 7 | *MW-EtOH | | | 10 | 75.5 | 351 | 0.79 ± 0.05 | 6.0 |
| 8 | | | | 20 | 76.1 | 351 | 0.56 ± 0.03 | 4.3 |

* Pressure for microwave extraction was automatically vented when reached 2.6 MPa. DFS, dry flax shives.

Trace amounts of phenolic compounds were detected for both microwave-water and microwave-EtOH extractions (results not shown). Noticeable amounts of free phenolics (0.3%) were observed with PLPW, PAA, and PAE (Buranov & Mazza, 2007; Buranov & Mazza, 2009).

The results of the chemical analysis of solid residues left after extraction are given in Table 3. Residues from PLPW contained the lowest hemicellulose content (1.7%) and more lignin (10.5%) compared to PAE (Table 3). This indicates that 90% of total hemicellulose and 54% of the lignin was removed with PLPW (Fig. 2). The solid residue from the PAE extraction contained 5.1% Klason lignin and 3.0% hemicellulose. Thus, extraction of flax shives with PAE removed 81% hemicellulose and 78% lignin (Fig. 2). Microwave extractions were not equally efficient in solubilising the hemicellulose and lignin biopolymers. Extraction with MW-Water removed only 10% of the total hemicellulose and 18% of the total lignin, whereas extraction with MW-EtOH removed 37% of both the total hemicellulose and 39% of the total lignin present in the flax shives. Longer extraction times (20 min in Table 1) reduced the HMH yield.

Under the experimental conditions used in this study, aqueous ethanol was efficient for the removal of both hemicellulose and lignin from flax shives with PAE and MW-EtOH extraction methods. However, PLPW was more selective and effective for the extraction of hemicellulose, but less suitable for the extraction of lignin. However, the differences between PAE and PLPW were relatively small. NaOH solution (0.5–2 M) has been reported to be able of solubilising up to 35.8% of total hemicelluloses from sugarcane bagasse at 55 °C (Sun et al., 2004).

The hemicellulose samples from all four processes (PLPW, PAE, MW-Water, and MW-EtOH) were acid-hydrolyzed to determine their constituent sugars and lignin contents (Sluiter et al., 2007).

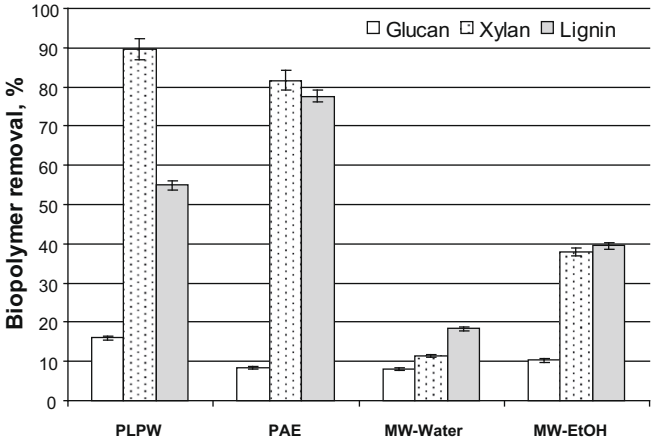


**Fig. 2.** Removal of biopolymers during extractions with pressurized low-polarity water (PLPW), pressurized aqueous ethanol (PAE), microwave-water (MW-Water), and microwave-ethanol (MW-EtOH).

**Table 2**
Yields of free sugars in the PLPW, PAE, MW-Water, and MW-EtOH liquid extracts (mg/g DFS).

| Extracts from | Xylose | Glucose | Galactose | Arabinose | Mannose | Total |
|---|---|---|---|---|---|---|
| PLPW | 34.6 ± 0.4 | 18.5 ± 0.3 | 15.1 ± 0.4 | 10.1 ± 0.3 | 23.8 ± 0.4 | 102.1 |
| PAE | 14.4 ± 0.3 | 13.9 ± 0.3 | 13.2 ± 0.3 | 6.5 ± 0.2 | 10.7 ± 0.3 | 58.7 |
| MW-Water | 1.4 ± 0.2 | 5.5 ± 0.2 | 4.3 ± 0.2 | 3.4 ± 0.2 | 3.5 ± 0.2 | 18.1 |
| MW-EtOH | 1.0 ± 0.1 | 4.9 ± 0.2 | 4.4 ± 0.2 | 3.0 ± 0.2 | 3.0 ± 0.2 | 16.3 |

**Table 3**
Composition of solid residues left after PLPW, PAE, MW-Water, and MW-EtOH extractions.*

| Samples | Glucan** (%) | Xylan (%) | Klason lignin (%) | Acid soluble lignin (%) | Ash (%) |
|---|---|---|---|---|---|
| Untreated flax shives | 32.5 ± 0.2 | 16.5 ± 0.5 | 22.4 ± 0.2 | 1.3 ± 0.1 | 3.3 ± 0.1 |
| PLPW | 27.4 ± 0.3 | 1.7 ± 0.1 | 10.5 ± 0.1 | 0.2 ± 0.01 | 2.1 ± 0.1 |
| PAE | 29.9 ± 0.3 | 3.0 ± 0.1 | 5.1 ± 0.1 | 0.2 ± 0.01 | 2.6 ± 0.1 |
| MW-water | 29.9 ± 0.4 | 14.7 ± 0.2 | 18.6 ± 0.3 | 0.7 ± 0.02 | 2.4 ± 0.1 |
| MW-EtOH | 30.6 ± 0.2 | 10.3 ± 0.2 | 13.9 ± 0.2 | 0.5 ± 0.02 | 2.1 ± 0.1 |

* Conditions for PLPW and PAE: 180 °C, 5.2 MPa, 3 mL/min, and 27 mL/g.
** Results are based on whole sample.

**Table 4**
Contents (%) of neutral sugars and lignins in high-molecular hemicelluloses from flax shives extracted with PLPW, PAE, MW-Water, and MW-EtOH.

| Constituent sugars (%) | PLPW | PAE | MW-Water (10 min) | MW-EtOH (10 min) |
|---|---|---|---|---|
| Glucose | 11.6 ± 0.2 | 8.6 ± 0.3 | 18.6 ± 0.3 | 8.9 ± 0.3 |
| Xylose | 42.5 ± 2.5 | 48.4 ± 2.5 | 46.9 ± 2.5 | 55.4 ± 2.5 |
| Galactose | 7.6 ± 0.3 | 4.7 ± 0.3 | 6.2 ± 0.3 | 4.5 ± 0.3 |
| Arabinose | 2.2 ± 0.1 | 0.9 ± 0.1 | 1.5 ± 0.1 | 0.9 ± 0.1 |
| Mannose | 3.9 ± 0.2 | 3.4 ± 0.2 | 4.2 ± 0.2 | 5.3 ± 0.2 |
| Total sugars | 68.8 | 66.0 | 77.4 | 75.0 |
| Klason lignin | 6.4 ± 0.3 | 25.8 ± 1.5 | 16.9 ± 1.1 | 11.1 ± 0.5 |
| Acid soluble lignin | 3.8 ± 0.2 | 1.9 ± 0.2 | 3.6 ± 0.2 | 2.0 ± 0.1 |
| Total lignin | 10.2 | 27.7 | 20.5 | 13.1 |

**Table 5**
Material balance for PLPW and PAE extractions.*

| Raw material and products | PLPW | PAE |
|---|---|---|
| Flax shives used (g) | 100 | 100 |
| Solid residue left (g) | 59.9 ± 2.2 | 51.1 ± 0.5 |
| High-molecular hemicelluloses (HMH) (g) | 9.7 ± 0.2 | 19.6 ± 0.5 |
| Lignin precipitated, (g) | 0.0 ± 0.0 | 9.8 ± 0.3 |
| Low-molecular hemicellulose (LMH) (g) | 15.2 ± 1.5 | 5.9 ± 0.3 |
| Total (g) | 84.8 | 86.4 |
| Unaccounted for (g) | 15.2 | 13.6 |

* Conditions for PLPW and PAE: 180 °C, 5.2 MPa, 3 mL/min, and 27 mL/g.

The results are presented in Table 4. The total sugar content in hemicelluloses was comparable and the major sugar component was xylose (~40–55%) in all cases. Glucose (~8–12%) was the second major sugar constituent, followed by galactose and mannose. Arabinose (~1–2%) was present in the lowest amount in all hemicelluloses. Similar sugar contents of xylans isolated from flax shives using microwave treatment have been reported by Jacobs et al. (2003). Slightly higher glucose content was observed with PLPW (11.6) and microwave-water (18.6%) which might be due to solubilisation of crystalline cellulose with hot water (Buranov & Mazza, 2007; Liu & Wyman, 2003). Additionally, a significant difference in the lignin content of the hemicellulosic fractions was detected. Hemicelluloses from PAE contained a high content lignin (27.7%), mostly Klason lignin. Hemicelluloses from PLPW had the lowest lignin content (10.2%) but also had increased arabinose (2.2%) and galactose (7.6%). Hemicellulosic fractions from wheat straw and sugarcane bagasse contained significant amounts of arabinose, confirming the presence of arabinoxylans (Sun et al., 2004).

The material balance for PLPW and PAE extractions, presented in Table 5, shows that PLPW yielded a higher lignin-rich solid residue, and a larger low-molecular weight hemicellulose fraction. PAE extracts, on the other hand, contained greater levels of high-molecular hemicelluloses and lignin.

The composition of the EtOH-soluble hemicelluloses was similar to the EtOH-insoluble hemicelluloses. LMH contained a lower quantity of Klason lignin (18.2%) and a higher quantity of acid-soluble lignin (4.3%); however, sugar contents were similar (Table 6 and Fig. 3).

The chemical composition of HMH from PLPW was different from that of PAE. The main difference between HMH from PLPW and PAE was the glucose content (11.1% and 6.2%) and lignin content (4.6% vs. 19.38%), respectively. The chemical composition of LMH from PLPW extract was similar with HMH and LMH from PAE extracts. LMH from both PLPW and PAE contained significant amount of Klason lignin (19.38 and 18.2%, respectively) and higher amounts of acid-soluble lignin (3.81 and 4.29%, respectively). Contents of other sugars were almost the same (Table 6). Lignin from PAE extracts contained traces of sugars.

### 3.2. Ion-moderated partition (IMP) chromatography

Ion-moderated partition (IMP) chromatography was applied to separate xylooligomers in the extracts according to their molecular size. The extracts from all four processes were further characterized to determine the xylooligomeric content. The results are given in Table 7 and showed that microwave extraction for shorter periods of time (10 min) produced considerably lower levels of xylooligomers (0.38–3.8 mg/g) than PLPW (25–52 mg/g) and PAE (10–27 mg/g). However, the yields of ethanol-precipitated HMH were much lower with microwave extractions (Table 1). Similar studies with corn stover at 200 °C for 10 min revealed that the total yield of xylooligomers, with degree of polymerization (DP) between 1–30, can reach up to 28% (Gray, Converse, & Wyman, 2007; Yang & Wyman, 2008). Extractions of hemicelluloses with ethanol-containing solvents (MW-EtOH and PAE) yielded higher levels of hemicelluloses and lower levels of xylooligomers compared to other methods (Table 7).

The major xylooligomers in all extracts were xylotriose (PLPW, 51.8 mg/g; PAE,27.3 mg/g; MW-Water, 3.8 mg/g and MW-EtOH,

**Table 6**
Composition of products recovered from PLPW and PAE extracts processed 180 °C, 5.2 MPa and a solvent/feed ratio of 27 mL/g.

| Constituent sugars (%) | Products from PLPW extracts | | Products from PAE extracts | | |
|---|---|---|---|---|---|
| | HMH | LMH | HMH | Lignin | LMH |
| Glucose | 11.1 ± 0.2 | 4.4 ± 0.2 | 6.2 ± 0.4 | 0.3 ± 0.1 | 4.41 ± 0.4 |
| Xylose | 42.3 ± 2.5 | 45.62 ± 2.5 | 47 ± 2.5 | 1.7 ± 0.3 | 45.16 ± 2.5 |
| Galactose | 5.6 ± 0.2 | 2.73 ± 0.2 | 3.65 ± 0.2 | 0.33 ± 0.1 | 3.97 ± 0.2 |
| Mannose | 6.1 ± 0.4 | 6.54 ± 0.4 | 5.15 ± 0.5 | 1.92 ± 0.2 | 5.62 ± 0.5 |
| Arabinose | 0.93 ± 0.2 | 0.72 ± 0.2 | 1.17 ± 0.3 | 0.03 ± 0.01 | 1.45 ± 0.3 |
| Total sugars | 66.03 | 60.01 | 63.17 | 4.28 | 60.61 |
| Klason lignin | 4.64 ± 0.3 | 19.38 ± 0.3 | 23.51 ± 2 | 87.7 ± 3 | 18.2 ± 0.9 |
| Acid soluble lignin | 3.23 ± 0.2 | 3.81 ± 0.2 | 2.1 ± 0.1 | 0.96 ± 0.2 | 4.29 ± 0.3 |
| Total lignin | 7.87 | 23.20 | 25.61 | 88.66 | 22.49 |

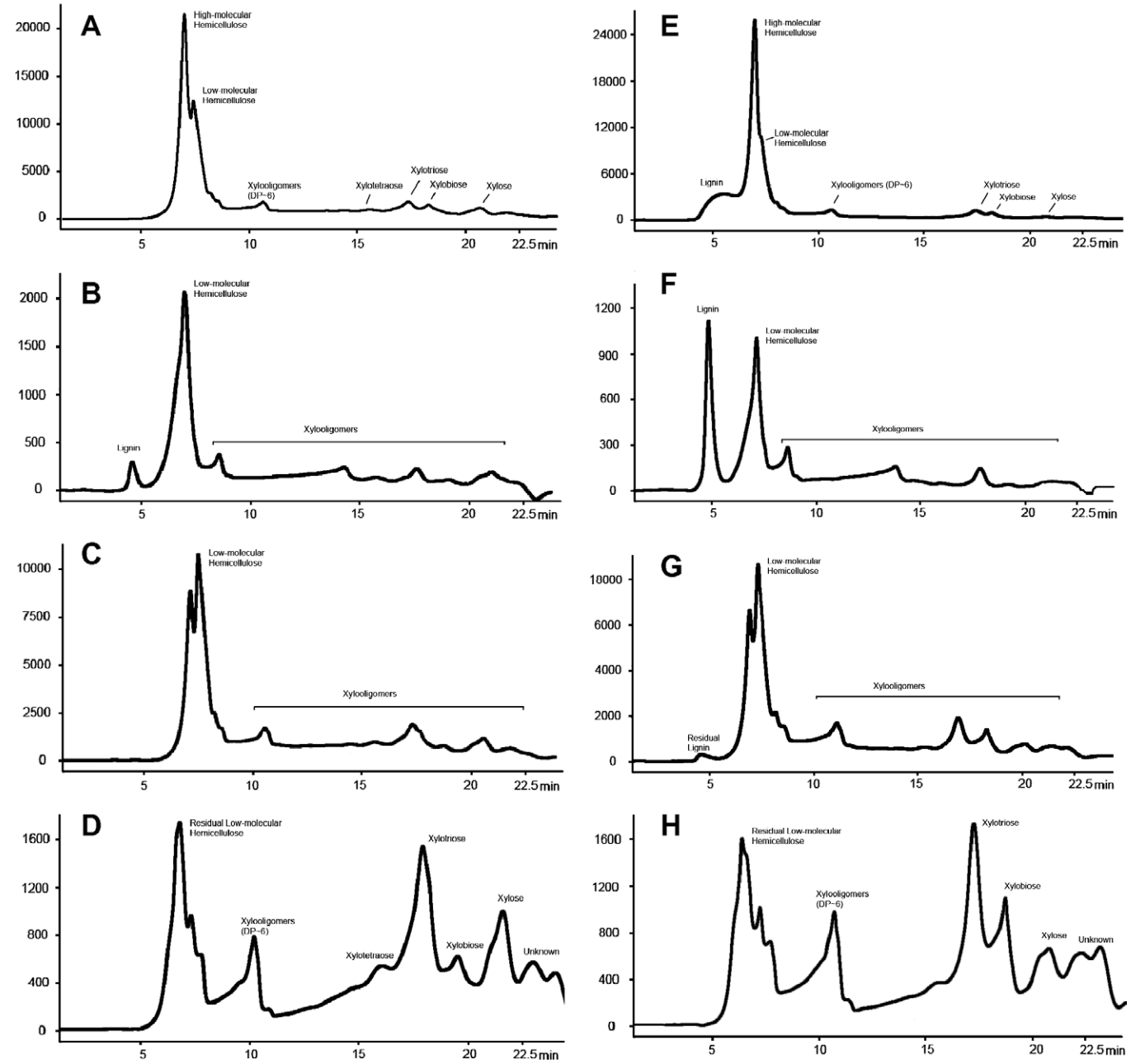


**Fig. 3.** Ion-moderated partition chromatograms of PLPW and PAE extracts (A and E), after hemicellulose removal via precipitation adding 4× vol. of 95% EtOH (B and F), after lignin precipitation in aqueous medium via the removal of EtOH (C and G) and after ultrafiltration (D and H).

**Table 7**
Analysis of extracts for xylooligosaccharides with IMP chromatography (mg/g DFS).

| Extraction methods | Xylose | Xylobiose | Xylotriose | Xylotetraose | Xylopentaose | HMH + LMH* (% peak area) |
|---|---|---|---|---|---|---|
| PLPW | 25.7 ± 0.3 | 28.6 ± 0.5 | 51.8 ± 1.2 | 25.4 ± 0.1 | 26.2 ± 0.8 | 100 |
| PAE | 10.0 ± 0.2 | 14.3 ± 0.4 | 27.3 ± 0.6 | 16.7 ± 0.1 | 13.5 ± 0.3 | 100 |
| MW-Water | 0.0 | 0.6 ± 0.1 | 3.8 ± 0.2 | 0.4 ± 0.0 | 3.5 ± 0.2 | 100 |
| MW-EtOH | 0.0 | 0.7 ± 0.1 | 5.0 ± 0.3 | 6.8 ± 0.1 | 0.6 ± 0.1 | 100 |
| *After precipitation with 4x vol. 95% Ethanol* | | | | | | LMH |
| PLPW | 27.8 ± 0.5 | 28.9 ± 0.5 | 49.1 ± 0.5 | 32.4 ± 0.1 | 23.2 ± 0.1 | 61.6 ± 0.2 |
| PAE | 13.0 ± 0.3 | 22.4 ± 0.1 | 24.6 ± 0.5 | 12.7 ± 0.1 | 11.6 ± 0.3 | 36.4 ± 0.1 |
| MW-Water | 0.0 | 0.3 ± 0.0 | 2.2 ± 0.1 | 0.5 ± 0.0 | 0.3 ± 0.0 | 44.5 ± 0.1 |
| MW-EtOH | 0.0 | 0.7 ± 0.1 | 4.9 ± 0.2 | 6.2 ± 0.1 | 0.0 | 42.2 ± 0.2 |

* Total peak area percentage for high molecular weight hemicelluloses (HMH) and low-molecular weight hemicelluloses (LMH) were assumed as 100% and after ethanol precipitation, the remaining LMH was calculated based on the percentage of peak area.

5.0 mg/g) followed by xylotetraose (16.7 mg/g), xylobiose (14.3 mg/g), and xylopentaose (13.5 mg/g) in PAE extracts. A similar order was detected in the extracts from microwave-ethanol. Aqueous extracts of PLPW contained higher quantities of xylobiose (28.6 mg/g), xylopentaose (26.2 mg/g), and xylotetraose (25.4%).

Xylose was not detected with microwave extractions, however, 10 and 25.7 mg/g of xylose was detected in the PAE and PLPW extracts, respectively. The xylooligomers content was not affected by ethanol precipitation, confirming their good solubility in both water and ethanol (Table 7 and Fig. 3B and F).

Precipitation of hemicelluloses with 4× vol. 95% ethanol removed only a portion of the HMH present in extracts (Fig. 3 and Table 7). In PAE extracts the content of remaining soluble hemicelluloses was 36.4%, and in PLPW extracts, the content was 61.6% which indicates that the yield of the LMH from PLPW is higher than that from PAE. Separation of high-molecular weight hemicelluloses, lignin and low-molecular weight hemicelluloses from PLPW and PAE extract was monitored with IMP chromatography (Fig. 3). IMP chromatograms of PLPW and PAE extracts (Fig. 3A and E), after high-molecular weight hemicelluloses precipitation with 4× vol. of 95% EtOH (Fig. 3B and F), after lignin precipitation in aqueous medium after EtOH removal (Fig. 3C and G), and after low-molecular weight hemicelluloses removal via ultrafiltration are illustrated in Fig. 3. As can be seen from the results, only high-molecular weight hemicelluloses can be precipitated using 4× vol. of 95% ethanol (Fig. 3A, B, E, and F). More than 61.6 and 36% of total hemicelluloses available in the original extracts was low-molecular weight hemicellulose which is soluble in ethanol. Lignin is also more soluble in ethanol (Fig. 3B and F). The removal of ethanol from PAE extracts resulted in the precipitation of lignin in aqueous medium and the yield was approximately 9% (Fig. 3C). No precipitation of lignin was observed with PLPW extracts, indicating a very low solubility of lignin in pure water. LMH was further separated from the aqueous phase using ultrafiltration (Fig. 3D and H). The low-molecular weight hemicelluloses yields from PLPW and PAE extracts were 15.2 and 5.9%, respectively (Table 5).

### 3.3. Molecular weight determined by size exclusion chromatography

The hemicellulose samples from the four processes (PLPW, PAE, MW-Water, MW-EtOH) were further studied to determine molecular weights with size exclusion chromatography (SEC). Solutions of hemicelluloses (2 mg/mL) were prepared in 0.1 M $NaNO_3$. Dextran standard samples with molecular weights of between 4400 and 750,000 Da were used to construct a calibration curve. Microwave heating was carried out for only 10 min. The molecular weight of high-molecular weight hemicelluloses decreased with an increase in microwave heating in both solvents; therefore, it is desirable to irradiate for only 10 min. Increasing microwave heating time from 10 min to 20 min resulted in lower molecular weights; therefore, the hemicellulose yields obtained with 20 min microwave heating were lower compared to heating for 10 min (Table 1). The molecular weight characteristics of hemicelluloses isolated with the four different processes are shown in Table 8. SEC chromatograms are illustrated in Figs. 4 and 5. The number-average ($M_n$), weight-average ($M_w$) molecular weights, and the molecular weight at highest point in the peak ($M_p$) of all four hemicellulose samples are close to each other (~10,000). Polydispersity ($M$w/$M$n) is the exception. The hemicelluloses extracted with microwave irradiation are more polydisperse (~4) than those from pressurized solvents (~1.74).

**Table 8**
Molecular properties of HMH and LMH extracted from flax shives using PLPW, PAE, MW-Water, and MW-EtOH.

| Extraction methods | Property | | | |
|---|---|---|---|---|
| | $M_w$ | $M_n$ | $M_p$ | $M_w/M_n$ |
| PLPW–HMH | 11,911 | 6858 | 10,179 | 1.74 |
| PAE–HMH | 11,069 | 7079 | 8191 | 1.56 |
| MW-Water–HMH | 32,251 | 8025 | 13,949 | 4.02 |
| MW-EtOH–HMH | 39,650 | 9950 | 13,114 | 3.98 |
| PLPW–LMH | 1752 | 433 | 1882 | 4.04 |
| PAE–LMH | 1467 | 356 | 1231 | 4.11 |

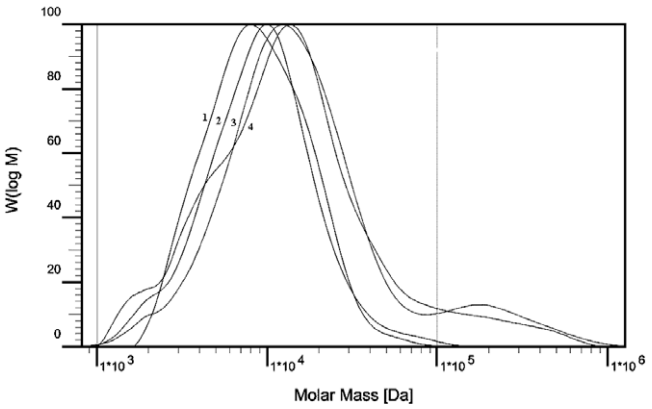


**Fig. 4.** SEC chromatograms of high-molecular hemicellulose samples: Curve 1 – hemicellulose from PAE; Curve 2 – hemicellulose from PLPW; Curve 3 – hemicellulose from MW-EtOH; Curve 4 – hemicellulose from MW-Water.

As shown in Table 8, microwave treatment with both water and aqueous ethanol favoured release of slightly higher molecular weight hemicelluloses as shown by their $M_w$ value of 32,251 and 39,650 g/mol, respectively. These values for hemicelluloses from PLPW and PAE were considerably lower being 11,911 and 11,069 g/mol, respectively. This clear difference suggests a possible effect of pressure on the molecular weights of analyzed hemicelluloses. Hemicelluloses extracted with aqueous ethanol had slightly lower, $M_p$ = 8191 for PAE and 13,114 for MW-EtOH, than those extracted with water, $M_p$ = 10,179 and 13,949, respectively.

Molecular weights ($M_w$) of hemicelluloses extracted from spruce with water under microwave irradiation, after impregnation with NaOH solution, were reported to be between 8000 and 80,000 Da (Lundqvist et al., 2002). The molecular weight of alkaline (0.5 M NaOH and 55 °C) extracted hemicelluloses from sugarcane bagasse was 45,370 Da and the addition of hydrogen peroxide (2%) decreased the $M_w$ to 23,340. Substantial degradation of hemicelluloses and reduction of molecular weights to 19,960 Da was observed under the stronger alkaline (2.0 M NaOH) and hydrogen

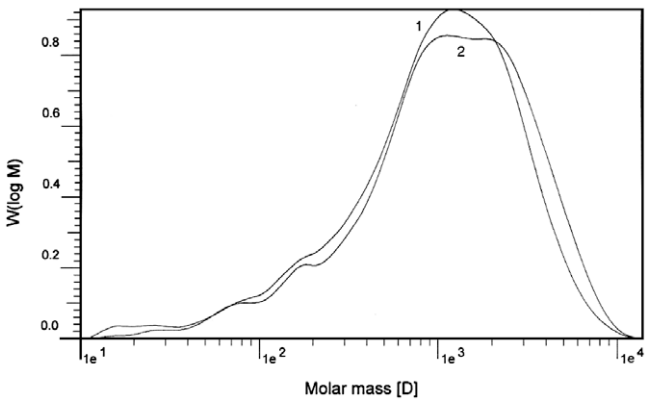


**Fig. 5.** SEC chromatograms of low-molecular hemicelluloses (LMH): Curve 1 – LMH from PAE; Curve 2 – LMH from PLPW.

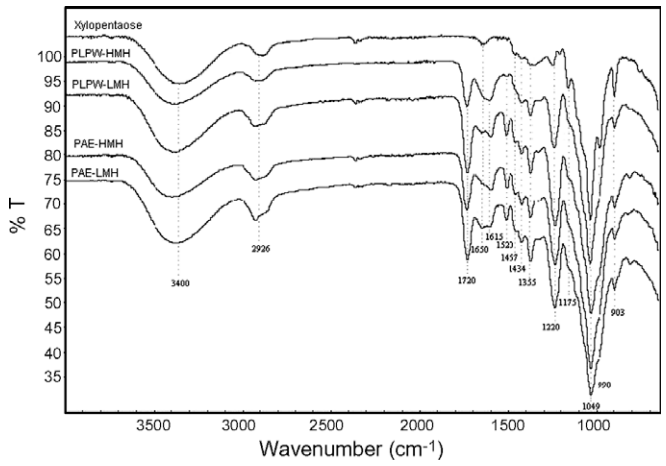


**Fig. 6.** Comparison of FT-IR spectra of high-molecular (HMH) and low-molecular hemicelluloses (LMH). PLPW–HMH – High-molecular hemicelluloses (HMH) from pressurized low-polarity water (PLPW) extract; PLPW–LMH – Low-molecular hemicelluloses (LMH) from pressurized low-polarity water (PLPW) extract; PAE–HMH – High-molecular hemicelluloses (HMH) from pressurized aqueous ethanol (PAE) extract; PAE–LMH – Low-molecular hemicelluloses (LMH) from pressurized aqueous ethanol (PAE) extract.

peroxide (3.0%) concentrations (Sun et al., 2004). The molecular weight of water-soluble hemicelluloses isolated from flax shives under microwave treatment was 4500 Da, which confirms the solubilisation of lower molecular weight hemicelluloses with water (Jacobs et al., 2003); however, during PLPW and PAE extraction of hemicelluloses from flax shives, the removal is almost (90 and 80%). This suggests that the extraction is followed by the cleavage of polymeric chain of xylans into lower molecular weight hemicellulosic polymers.

Size exclusion chromatography for EtOH-soluble low-molecular waight hemicelluloses revealed a different picture (Fig. 5). The molecular weight of soluble hemicellulose was found to be around $M_w$ = 1752 g/mol, $M_n$ = 433 g/mol, $M_p$ = 1882, and $M_w/M_n$ = 4.1. These data are appreciably lower than those for ethanol-precipitated hemicelluloses as shown in Table 8.

### 3.4. FT-IR spectra of hemicelluloses

The FT-IR spectra of HMH and LMH were taken for the comparison of their structures. The FT-IR spectra are illustrated in Fig. 6. The FT-IR spectra of hemicelluloses showed that they contain all the specific signals similar to those reported by Sun et al. (2004). The specific band maximum at 1220 $cm^{-1}$ for hemicellulose is clearly illustrated in both xylan samples. The bands between 1175 and 1000 $cm^{-1}$ are typical of xylans and reflect the stretching and bending vibrations of C–O, C–C, C–OH, and C–O–C with a broad intense signal at 1049 $cm^{-1}$. The presence of arabinosyl side chains is indicated by low-intensity shoulders at 1175 and 990 $cm^{-1}$ on both spectra (Kacuraková, Belton, Wilson, Hirsch, & Ebringerová, 1998). In both spectra the signal for the C–O stretching vibrations of acetyl, uronic, and ferulic ester groups gave intense signal at 1720 $cm^{-1}$. The intense signal at 1650 $cm^{-1}$ is due to absorbed water. The glycosidic linkage gave a small signal at 903 $cm^{-1}$. Bands at 1467 and 1434 $cm^{-1}$ were due to the $-CH_2$ stretching vibrations. The intense band at 1520 $cm^{-1}$ indicated the small amount of lignin present in hemicelluloses. The intense band for the C–H stretching vibrations was observed at 2926 $cm^{-1}$. The prominent band around 3400 $cm^{-1}$ represents the O–H stretching vibrations and hydrogen bonding. There was no difference in the FT-IR spectra between the two hemicelluloses isolated using different processes except for their molecular weights.

## 4. Conclusions

Removal of hemicelluloses from biomass is high with PLPW (90%) and PAE (80%), and low with microwave-water and microwave-ethanol processes (19 and 40%). Increasing the time of microwave irradiation leads to a reduction of hemicelluloses yield, probably due to the degradation of macromolecular xylan. High-molecular weight hemicelluloses isolated from PAE extracts contained ~23% lignin, while the PLPW extracts contained ~5% lignin. Low-molecular weight hemicelluloses separated by ultrafiltration from PLPW and PAE extracts contained similar amounts of lignin (~20%). However, the low yield of low-molecular weight hemicelluloses from PLPW was higher (~15%) compared to that from PAE (~6%). The PAE extracts contained less xylooligomers than the PLPW extracts, but the molecular weight of the hemicelluloses extracted by these two processes were practically identical. Longer extraction times reduced the molecular weights of the extracted hemicelluloses.

## Acknowledgements

The authors are grateful to the ABIP program of AAFC and the Canada Biomass Innovation Networks program of NRCan for partial funding of this work. We also thank Lana Fukumoto and David Godfrey for technical assistance, and Dr. Gordon Neish for editorial input.

## References

Bobleter, O. (1994). Hydrothermal degradation of polymers derived from plants. *Progress in Polymer Science, 19*, 797–841.
Buranov, A. U., & Mazza, G. (2007). Fractionation of flax shives by water and aqueous ammonia treatment in a pressurized low-polarity water extractor. *Journal of Agricultural and Food Chemistry, 55*, 8548–8555.
Buranov, A. U., & Mazza, G. (2008). Lignin in straw of herbaceous crops (Review). *Industrial Crops and Products, 28*, 237–259.
Buranov, A. U., & Mazza, G. (2009). Extraction and purification of ferulic acid and vanillin in flax shives and wheat bran by alkaline-hydrolysis and pressurized solvents. *Food Chemistry, 115*, 1542–1548.
Cox, M., El-Shafey, E., Pichugin, A. A., & Appleton, Q. (1999). Preparation and characterization of a carbon adsorbent from flax shive by dehydration with sulfuric acid. *Journal of Chemical Technology and Biotechnology, 74*, 1019–1029.
Ebringerová, A., Hromádková, Z., Kacuráková, M., & Antal, M. (1994). Quaternized xylans: Synthesis and structural characterization. *Carbohydrate Polymers, 24*, 301–308.
Fang, J. M., Sun, R. C., Fowler, P., Tomkinson, J., & Hill, C. A. S. (1999). Esterification of wheat straw hemicelluloses in the *N,N*-dimethylformamide/lithium chloride homogeneous system. *Journal of Applied Polymer Science, 74*, 2301–2311.
Gabrielii, I., Gatenholm, P., Glasser, W. G., Jain, R. K., & Kenne, L. (2000). Separation, characterization and hydrogel-formation of hemicellulose from aspen wood. *Carbohydrate Polymers, 43*, 367–374.
Geerdes, J. D., & Smith, F. J. (1955). The constitution of the hemicellulose of the straw of flax (*Linum usitatissimum* Sp.). II. Hydrolysis of the methylated hemicellulose. *Journal of American Chemical Society, 77*(357), 2–3576.
Gray, M. C., Converse, A. O., & Wyman, C. E. (2007). Solubilities of oligomers mixtures produced by the hydrolysis of xylans and corn stover in water at 180 °C. *Industrial Engineering and Chemistry Research, 46*(8), 2383–2391.
Grohmann, K., Torget, R. W., & Himmel, M. E. (1985). Optimization of dilute acid pre-treatment of biomass. *Biotechnology and Bioengineering, 15*, 59–80.
Jacobs, A., Palm, M., Zacchi, G., & Dahlman, O. (2003). Isolation and characterization of water-soluble hemicelluloses from flax shive. *Carbohydrate Research, 338*, 1869–1876.
Jain, R. K., Sjöstedt, M., & Glasser, W. (2000). Thermoplastic xylan derivatives with propylene oxide. *Cellulose, 7*, 319–336.
Kacuraková, M., Belton, P. S., Wilson, R. H., Hirsch, J., & Ebringerová, A. (1998). Hydration properties of xylan-type structures: an FT-IR study of xylooligosaccharides. *Journal of the Science of Food and Agriculture, 77*, 38–44.
Karimi, K., Kheradmandinia, S., & Taherzadeh, M. (2006). Conversion of rice straw to sugars by dilute-acid hydrolysis. *Biomass and Bioenergy, 30*, 247–253.
Lawther, J. M., Sun, R. C., & Banks, W. B. (1995). Extraction, fractionation, and characterization of structural polysaccharides from wheat straw. *Journal of Agricultural and Food Chemistry, 43*, 667–675.

Li, X., Converse, A. O., & Wyman, C. E. (2003). Characterization of molecular weight distribution of oligomers from autocatalyzed batch hydrolysis of xylan. *Applied Biochemistry and Biotechnology, 105–108*, 515–522.

Liu, C., & Wyman, C. E. (2003). The effect of flow rate of compressed hot water on xylan, lignin, and total mass removal from corn stover. *Industrial Engineering and Chemical Research, 42*, 5409–5416.

Lundqvist, J., Teleman, A., Junel, L., Zacchi, G., Dahlman, O., Tjerneld, F., et al. (2002). Isolation and characterization of galactoglucomannan from spruce (*Picea abies*). *Carbohydrate Polymers, 48*, 29–39.

Mazza, G., & Cacace, J. E. (2005). Extraction of phytochemicals. US Patent Application. Pub. No. US 2007/0014912 A1, Pub. Date: Jan 18, 2007; Filing Date: May 13, 2005, published by United States Patent and Trademark Office, Washington, DC (Patent).

McDougall, G. J. (1993). Isolation and partial characterisation of the non-cellulosic polysaccharides of flax fibre. *Carbohydrate Research, 241*, 227–236.

Preziosi-Belloy, L., Nolleau, V., & Navarro, J. M. (1997). Fermentation of hemicellulosic sugars and sugar mixtures to xylitol by *Candida parapsilosis*. *Enzyme and Microbial Technology, 21*, 124–129.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., et al. (2007). *Determination of structural carbohydrates and lignin in biomass. Laboratory Analytical Procedure*. National Renewable Energy Laboratory, NREL: Golden, Co..

Sun, R. C., Lawther, J. M., & Banks, W. B. (1996). Fractional and structural characterization of wheat straw hemicelluloses. *Carbohydrate Polymers, 29*, 325–331.

Sun, J. X., Sun, X. F., Sun, R. C., & Su, Y. Q. (2004). Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses. *Carbohydrate Polymers, 56*, 195–204.

Teramoto, Y., Tanaka, N., Lee, S.-H., & Endo, T. (2008). Pretreatment of eucalyptus wood chips for enzymatic saccharification using combined sulfuric acid-free ethanol cooking and ball milling. *Biotechnology and Bioengineering, 99*(1), 75–85.

Torget, R., Walter, P., Himmel, M., & Grohmann, K. (1991). Dilute-acid hydrolysis pre-treatment of corn residues and short-rotation woody crops. *Applied Biochemistry and Biotechnology, 28*(29), 75–86.

Van Hazendonk, J. M., Reinerink, E. J. M., Waard, P. D., & van Dam, J. E. G. (1996). Structural analysis of acetylated hemicellulose polysaccharides from fibre flax (*Linum usitatissimum* L.). *Carbohydrate Research, 291*(14), 1–154.

Yang, B., & Wyman, C. E. (2008). Characterization of the degree of polymerization of xylooligomers produced by flowthrough hydrolysis of pure xylan and corn stover with water. *Bioresource Technology, 99*, 5756–5762.