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Production of Carbohydrates, Lignins, and Minor Components from Triticale Straw by Hydrothermal Treatment

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ABSTRACT: The effects of temperature (116 °C, 150 °C, and 183 °C) and flow rate (66, 150, and 234 mL/min) on the fractionation of triticale straw into different products was determined using a flow-through pressurized low polarity water reactor. The greatest concentration of biomass was hydrolyzed and extracted in the first two of eight 600 mL fractions (1.2 L), after which dry matter yield decreased. Carbohydrate, lignin, acetyl group, and uronic acid yield increased with temperature, but there was no effect due to flow rate. Most dry matter extracted at 116 °C was probably associated with the extractives. Xylan yields decrease slightly at the highest flow rate due to a decrease in the residence time of the acids produced in situ. Carbohydrates were extracted mostly as oligosaccharides, and the highest processing temperature resulted in the production of furans from the xylose and arabinose in the liquid extracts.

KEYWORDS: Hemicellulose, lignin, lignocellulosic biomass, bioproducts, biochemicals, pressurized water, hydrolysis, furans

INTRODUCTION

As worldwide energy demand continues to grow, and the negative environmental impacts of fossil fuel use becomes increasingly evident, interest in obtaining energy and materials from renewable resources is also growing; consequently, much attention has been focused on the utilization of lignocellulosic biomass as a raw material to supply our energy and chemical needs.[1,2]

Lignocellulosic biomass consists primarily of cellulose (30−50%), hemicellulose (15−35%), and lignin (10−30%), with lesser amounts of ash, oils, waxes, and other components.[3−5] Fractionation using various physical, biological, thermal, or chemical methods allows for improved utilization of the biomass by separating the components into different processing streams. Each stream may then be treated separately for the production of biofuels, biochemicals, or bioproducts. Fractionation can be accomplished in a variety of ways, including direct enzymatic conversion of the raw material, acid hydrolysis, or through hydrothermal treatment with pressurized low polarity water (PLPW). When water is heated under pressure from 25 °C to 200 °C its dielectric constant (polarity) decreases from 79 to 35, reaching values similar to solvents such as ethanol (24) or methanol (33).[6]

Currently, thermochemical fractionation is more suitable for commercial application than mechanical and biological methods because of shorter processing times, higher yields, limited chemical use, and lower energy requirements.[7] The benefits of hydrothermal treatment with PLPW over other popular thermochemical methods such as dilute acid treatments are as follows: the low cost of water relative to other solvents or chemicals; reduced corrosion of processing equipment; lower concentrations of sugar degradation products produced during processing (although these products could be a benefit); no generation of waste streams from neutralization of extracts, which may add to the processing costs; use of a nontoxic processing medium allows for the production of food grade products and pharmaceuticals.

Carbohydrates, lignins, and other components produced from the hydrothermal treatment of lignocellulosic biomass can be used as the basis for providing raw materials for a wide range of bioproducts. There is considerable information on the hydrothermal processing of agricultural residues, but the composition of the product streams is dependent on the starting material.[8−11] For instance, xylo-oligosaccharides derived from plant hemicellulose are bioactive molecules, which have a wide range of reported applications.[12,13] Many of the benefits are dependent on the molecular weight and nature of the substituents attached to the xylo-oligosaccharides, which will also depend on the hydrothermal processing conditions.[12] Hydrothermal treatment has been successful at producing a wide range of xylo-oligosaccharides from a variety of crop material including rice husks,[11] oat spelt,[14] flax shives,[15] brewery's spent grain,[16] corn cobs,[16] wheat bran,[16] and *Eucalyptus* wood.[16] Most of these studies occurred in batch systems, and it has been shown that the reactions in a flow-through reaction system can be quite different. Flow-through reactors have been shown to remove more hemicellulose and lignin, with fewer degradation products forming than in a batch system.[10]

Previously we have examined the optimization of hydrothermal treatment with PLPW of triticale straw for hemicellulose yield and residue susceptibility to enzymatic hydrolysis.[17] There has been interest in the use of triticale as an industrial crop because triticale yields more grain and produces more biomass (straw) than other cereal crops.[18] As such, utilization of triticale can increase the available biomass for industrial use without increasing competition with food production for agricultural land. The focus of this work is on the hydrothermal treatment of triticale straw in a flow-through reactor and the effects of temperature and flow rate on the production of carbohydrates, lignins, and other minor components. Mass balances and composition of the liquid extracts during the course of the hydrothermal treatment of triticale straw with PLPW were determined to examine key trends and materials that may be of interest for bioproduct and biochemical production.

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MATERIALS AND METHODS

Materials. Triticale straw (x *Triticosecale* cv. AC Ultima), from the 2008 crop year, grown near Indian Head, SK, Canada, was obtained from Agriculture and Agri-Food Canada's Semiarid Prairie Agricultural Research Centre, Swift Current, SK. Samples were coarsely ground in a Model SM 2000 Retsch mill (Retsch GmbH, Haan, Germany) to pass through an 8 mm discharge screen. The ground sample was then sieved for 5 min in a Model AS200Tap tapping sieve shaker (Retsch GmbH, Haan, Germany) equipped with a No. 10 sieve (2 mm size) to separate out long pieces of straw. This process segregates the particles according to the smallest dimensions which pass through the sieve. The resulting sample, which passed through the 2 mm sieve, contained some fine particles as well as some pieces of straw up to 10 mm in length. This particle distribution should not adversely affect the hydrolysis because the wall thickness of the straw is on the order of 0.2 mm, which should be the controlling dimension for the process. The ground sample was bagged and stored at −20 °C prior to use.

PLPW Fractionation and Extraction. The fixed bed flow-through reactor system used for the PLPW extractions has been described previously.[17] A 126 g sample (approximately 120 g of dry matter) of coarsely ground triticale straw was uniformly packed into a stainless steel flanged column 500 mm long × 50 mm i.d. (MODcol) (Mandel Scientific Company Inc., Guelph, ON) to a bed depth of 400 mm. To keep the sample inside the reaction vessel, and to help promote dispersion of the solvent, both ends were packed with 50 mm of stainless steel wool and capped with a 20 and 100 μm stainless steel frit at the inlet and outlet, respectively.

The hydrothermal treatment procedure was initiated by first flooding the reaction vessel with a small amount of water (equivalent to the void volume, < 300 mL) and then warming the system to the experimental temperature (116 °C, 150 °C, or 183 °C) and holding it there for 1 h to allow the temperature of the sample to equilibrate within the column before commencing the flow through the system with the pump and adjusting the back pressure regulator to 11 MPa. There was no indication that significant reaction occurred during the preheat phase before the initiation of flow within the reactor, probably because the temperature was below the target and the availability of solvent was limited. Upon commencement of flow through the system (66, 150, or 234 mL/min corresponding to a superficial velocity in the reactor of 0.034, 0.077, or 0.119 m/min, respectively) the first portion of solution, which contained no analyte (corresponding to the dead volume in the system from the top of the reaction vessel to the collection vessel), was discarded and the predetermined volume of solution (4.8 L total; 8 × 600 mL fractions) was collected based on the chosen solvent-to-solid ratio of 40 mL of solvent for every 1 g of starting dry matter. After each experiment, the system was flushed with PLPW for 1 h to remove any residue. Extracts collected from each experiment were stored at −20 °C, and the solid residues were removed from the reaction vessel, freeze-dried, and stored at −20 °C until they were analyzed.

Compositional Analysis. *Solid Residues.* Solid residues were analyzed for structural carbohydrates, lignin, acetyl groups, and ash content according to NREL (National Renewable Energy Laboratory) standard analytical procedures.[19,20]

Acid insoluble lignin (AIL) and acid soluble lignin (ASL) were determined by first hydrolyzing samples with 72% sulfuric acid for 1 h at 30 °C in a water bath and then diluting to 4% sulfuric acid and autoclaving at 121 °C for 1 h in sealed glass pressure tubes. AIL was analyzed gravimetrically after the hydrolysis of the cellulose and hemicellulose.[20] ASL in the hydrolysate was determined by the spectrophotometric method at 320 nm.[19] An absorptivity of 30 L g^{-1} cm^{-1} was used to convert absorbance readings to mass values. The results for lignin content of the samples are reported as the sum of the AIL and ASL.

Structural carbohydrates, cellulose (glucose) and hemicellulose (xylose, galactose, arabinose, and mannose) in the solid residues were determined quantitatively from hydrolysate obtained from the lignin analysis by HPLC using an Agilent 1100 equipped with a refractive index detector (Agilent Technologies, Palo Alto, CA). The HPLC analysis was carried out using a 300 mm × 7.8 mm i.d. Bio-Rad Aminex HPX-87P column equipped with a Micro-Guard deashing guard cartridge (Bio-Rad Laboratories, Hercules, CA) operating at 75 °C. The HPLC system consisted of a G1329A autosampler and G1312A delivery system that were controlled by Agilent Chemstation Plus software (Agilent Technologies, Palo Alto, CA). HPLC grade filtered water (Milli-Q) was used as the mobile phase at a flow rate of 0.5 mL/min and, for each sample, 50 μL of prefiltered aliquot was injected automatically. The carbohydrate concentrations were determined by comparison against a set of known sugar standards and the application of a sugar recovery factor according to NREL standard procedures.[20]

Acetyl groups and formic and levulinic acids were quantitatively measured from the hydrolysate with HPLC using an Agilent 1100 equipped with a refractive index detector (Agilent Technologies, Palo Alto, CA).[20] The HPLC analysis was conducted using a 300 mm × 7.8 mm i.d. Biorad Aminex HPX-87H column with a 30 mm × 4.6 mm i.d. Cation H refill Cartridge guard column (Bio-Rad Laboratories, Hercules, CA) operating at 55 °C with a 0.005 M H_2SO_4 mobile-phase at a flow rate of 0.6 mL/min.

Ash content of the solids was determined by complete combustion of the samples in a model F-A1730 muffle furnace (Thermolyne Corporation, Dubuque, IA) equipped with a Furnatrol II series 413 temperature controller (Thermolyne Corporation, Dubuque, IA). The temperature controller was set to ramp up to 105 °C from room temperature, held for 12 min, ramped up to 250 at 10 °C/min, held for 30 min, ramped up to 575 at 20 °C/min, held for 180 min, dropped to 105 °C, and held until the sample was removed. The remaining residue in the crucible was taken as the ash content.

Uronic acids in the hydrolysate were quantified by the Scott method.[21] An aliquot (0.125 mL) of the hydrolysate was added to 0.125 mL of 2% NaCl−3% H_3BO_3 solution in a test tube. Concentrated H_2SO_4 was added to the test tube in an ice bath and mixed. The test tube was then heated for 40 min at 70 °C in a water bath. The test tubes were then removed and allowed to cool to room temperature before 0.1 mL of 0.1% 3,5-dimethylphenol in glacial acetic acid was added to the reactant. After 10 min, the uronic acids concentration was determined by averaging the absorbance at 400 and 450 nm and comparing it to a standard curve of D-glucuronic acid (Sigma-Aldrich Co., St. Louis, MO).

Protein content was estimated from the nitrogen content of the samples.[22] Prior to analysis, the solid residues were ground in a MF 10 hammer mill (IKA-Werke GmbH & Co. KG, Staufen, Germany) to pass through a 0.5 mm discharge screen. Samples were dried overnight in a vacuum oven at 60 °C prior to analysis. Nitrogen content was determined by combusting the dried samples at 850 °C using a Leco FP-528 nitrogen analyzer (Leco Corporation, St. Joseph, MI). A standard curve for nitrogen was produced using ethylenediaminetetraacetic acid (EDTA) and corn flour (Leco Corporation, St. Joseph, MI). Protein content was estimated by multiplying the nitrogen content (%) by a factor of 6.25.

Liquid Extracts. Liquid extracts were analyzed for structural carbohydrates and degradation products according to NREL standard analytical procedures.[23] Structural carbohydrates, cellulose (glucose), and hemicellulose (xylose, galactose, arabinose, and mannose) in the liquid extracts were determined by hydrolyzing the liquid extracts with 4% sulfuric acid and autoclaving at 121 °C for 1 h in sealed glass pressure tubes. Samples were neutralized with calcium carbonate and filtered through a 0.20 μm membrane and analyzed with the same equipment as the solid residues. The total structural carbohydrate content for the samples included both monomers and hydrolyzed oligosaccharides.

A subsample of each liquid extract was neutralized with calcium carbonate, filtered through a 0.20 μm syringe filter, and used for direct HPLC determination of carbohydrate monomers. The concentration of carbohydrate oligosaccharides was then calculated by taking the difference between the hydrolyzed total carbohydrate content and the

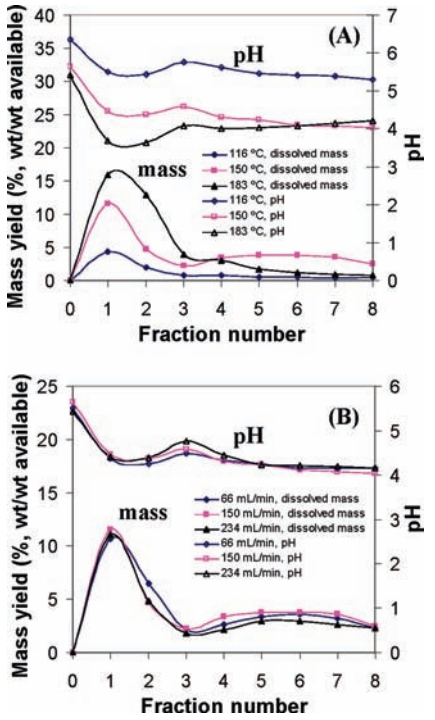

Figure 1. Total dissolved mass and pH of liquid fractions (600 mL) from PLPW extraction of triticale straw with a solvent-to-solid ratio of 40 mL/g at (A) constant flow rate of 150 mL/min and (B) constant temperature of 150 °C.

nonhydrolyzed monomer content. The degradation products 5-hydroxy-2-methylfurfural (HMF) and furfural were determined from the same sample by direct HPLC determination using DAD detection.

A portion of the liquid extracts were freeze-dried and the resulting solids were analyzed for lignin, acetyl groups, uronic acids, ash, and protein contents using the procedures described for the solid residues.

RESULTS AND DISCUSSION

PLPW Fractionation. The greatest amount of biomass was hydrolyzed, solubilized, and extracted during the first two 600 mL fractions (1.2 L), after which the recovery of dry matter decreased considerably (Figure 1). Dry matter recovery and pH were affected by temperature, with the greatest dry matter recovery at the highest temperature, which also corresponded to the lowest fractional pH (Figure 1A). Early fractions at 183.6 °C had a pH of 3.6 when measured at room temperature. At 150 °C this value increased to 4.4, and at 116.4 °C the pH increased to 5.4. This may be a consequence of the autohydrolysis process, which is the result of the catalytic action of hydronium ions from the production of in situ acids, such as acetic acid. The amount of acetic acid produced is dependent on the duration and severity of the processing conditions.[24] In these experiments the decrease in pH was due to the increase in acetic acid production at higher temperatures, which in turn aided in the hydrolysis process and dry matter extraction. At 116.4 °C, 0.13 g of acetyl groups, which consists of acetic acid and acetyl group oligosaccharides, was released from the biomass over the first two fractions. At 150 °C the production of acetyl groups increased to 0.51 g, and at 183.6 °C it increased to 0.88 g. There was no difference in acetyl group production, dry matter extraction, and pH over the first two fractions due to flow rate at a constant temperature (Figure 1B). As such, there seems to be a connection between acetyl group production and dry matter extraction, suggesting that acetic acid released from the autohydrolysis process may play a key role in the kinetics of the reaction.

There was an increase in dissolved mass with increasing temperature, but flow rate had little effect on dissolved mass (Table 1). There was a slight decrease in the dissolved mass at the highest flow rate of 234 mL/min. This is likely due to the reduction in residence time of the PLPW and in situ produced acetic acid within the reactor. Overall, the material balance for the hydrothermal treatment was good, with less than 4% of the starting dry matter lost. At 183.6 °C losses were just over 10% of the original dry matter in the reactor, but this condition also yielded the lowest solid residue remaining and the highest amount of dissolved mass in the liquid extracts. Most of these losses were in the xylan fraction, where nearly 40% was unaccounted for, yet there is not a large enough increase in furfural yields to account for this. It is possible that a small portion of the biomass, specifically the hemicellulose fraction containing the xylan, underwent some form of pyrolysis or torrefaction during the hydrolysis, resulting in a portion of the product converting to gaseous form. Hemicellulose is much easier to degrade thermally than the cellulose and lignin fractions due to the susceptibility of pentosans to hydrolysis reactions.[25] Pyrolysis in PLPW is similar to pyrolysis in air, with the exception of hydrolysis reactions and solubilization of the products in the reaction medium.[26] The losses to pyrolysis are not likely to be large, only 3% of solid waste was converted to gaseous products at 230 °C,[25] but they do help to account for some of the missing mass at 183.6 °C, and this supposition is supported by the presence of some char-like solids in the dried extracts. The material that is unaccounted for in the residue and liquid extracts could also be a variety of materials not measured, including lipophilic compounds, phenolics, organic acids, and other degradation products. Of these, waxes and phenolics are the most abundant, and triticale straw has been shown to contain up to 1% wax[27] and 0.35% phenolic compounds.[28]

Composition of the Solid Residues and Liquid Extracts. *Cellulose.* Composition of the native triticale straw and the solid and liquid fractions from the hydrothermal treatment are presented in Table 2. All processing conditions enriched the glucan content of the solid residue. Preserving glucan in the solid residues is desirable for subsequent enzymatic hydrolysis to maximize the glucose for ethanol production.[17] Cellulose is a high molecular weight linear polymer of β-1,4-linked D-glucose units, which has a configuration that promotes the ordering of the polymer chains into tightly packed, highly crystalline structures that are insoluble in water and are resistant to hydrolysis. Yield of glucose and gluco-oligosaccharides (total glucose) from the hydrothermal treatment was less than 10% for all processing temperatures (Figure 2). Content of total glucose in the liquid extracts remained low for most processing conditions (Figures 3 and 4). At 116 °C, conditions in the reactor were not severe enough to hydrolyze the cellulose, so some of the total glucose extracted probably existed as free sugars. At 150 °C and 183 °C, the fractional content of total glucose increased (Figure 3A), but overall yield was similar at the two temperatures (Figure 2). There was a small increase in the amount of glucose monomers at 183 °C, and the proportion of glucose monomers increased over those obtained at 150 °C (Table 2). Low total glucose content in the liquid extracts suggests that most of the total glucose was associated with the hemicellulose or extractives and not with the cellulose fraction of the biomass. Cellulose hydrolysis is low in

Table 1. Material Balance for PLPW Fractionation of Triticale Straw

	processing conditions					
raw material and products	116 °C[a]	150 °C[a]	183 °C[a]	66 mL/min[b]	150 mL/min[b]	234 mL/min[b]
starting material (g)	120.00	120.00	120.00	120.00	120.00	120.00
solid residue (g)	105.13	71.82	58.95	73.49	71.82	77.30
total dissolved mass (g)	12.08	43.48	48.92	42.35	43.48	38.53
total (g)	117.21	115.30	107.87	115.84	115.30	115.83
unaccounted material (losses)[c] (g)	2.79	4.70	12.13	4.16	4.70	4.17

[a] Constant flow rate (150 mL/min) and solvent-to-solid ratio (40 mL/g). [b] Constant temperature (150 °C) and solvent-to-solid ratio (40 mL/g). [c] Calculated as starting material − solid residue − dissolved mass.

Table 2. Carbohydrates, Lignin, and Minor Components in Solid Residue and Freeze-Dried Liquid Fractions of Triticale Straw after PLPW Fractionation and Extraction

		processing conditions					
constituents (%)	native material	116 °C[a]	150 °C[a]	183 °C[a]	66 mL/min[b]	150 mL/min[b]	234 L/min[b]
		Solid Residue					
glucan	36.28	40.75	54.91	68.39	53.29	54.91	50.85
xylan	21.01	22.44	12.41	2.58	112.411	12.41	13.09
galactan	1.21	1.14	0.50	0.54	0.60	0.50	0.69
arabinan	2.12	2.09	0.47	0.12	0.47	0.47	0.64
mannan	0.42	0.23	0.26	0.16	0.30	0.26	0.31
lignin	16.19c	17.07[d]	16.63[d]	16.92[d]	18.50[d]	16.63[d]	17.28[d]
acetyl groups	1.82	2.31	1.10	0.17	1.12	1.10	1.34
uronic acid	1.67	1.71	0.70	0.39	0.58	0.70	0.76
protein	4.39	3.21	3.57	3.20	3.34	3.57	3.88
ash	7.51	4.11	5.67	4.61	5.39	5.67	5.72
others (by difference, 100%)	7.38	4.94	3.78	2.92	5.30	3.78	5.44
		Liquid Fractions (total dissolved mass)					
gluco-oligosaccharides (GO)		6.01	6.98	4.06	5.99	6.98	7.69
xylo-oligosaccharides (XO)		6.16	34.46	18.68	34.78	34.46	27.37
galacto-oligosaccharides (GOS)		2.70	2.45	0.79	2.15	2.45	2.31
arabino-oligosaccharides (ArO)		1.60	3.11	0.61	2.82	3.11	3.09
manno-oligosaccharides (MOS)		1.04	0.72	0.12	0.67	0.72	0.78
glucose		1.35	0.67	1.40	0.68	0.67	0.78
xylose		0.54	0.74	3.80	1.86	0.74	1.52
galactose		1.58	0.28	1.23	0.45	0.28	0.41
arabinose		0.98	2.21	1.69	2.29	2.21	2.42
mannose		0.22	0.09	0.98	0.26	0.09	0.08
HMF		0.00	0.03	0.58	0.02	0.03	0.02
furfural		0.00	0.23	4.46	0.28	0.23	0.22
lignin		9.06[d]	12.52[d]	26.01[d]	13.23[d]	12.52[d]	12.87[d]
acetyl groups		1.44	2.99	2.39	3.13	2.99	2.89
uronic acid		0.80	1.94	0.43	2.19	1.94	1.73
formic acid		1.20	1.39	1.12	0.97	1.39	1.12
levulinic acid		0.61	0.41	0.56	0.26	0.41	0.42
protein		15.10	7.65	6.39	6.89	7.65	8.24
ash		26.27	11.09	11.21	10.56	11.09	12.30
others (by difference, 100%)		23.34	10.04	13.50	10.52	10.04	13.74

[a] Constant flow rate (150 mL/min) and solvent-to-solid ratio (40 mL/g). [b] Constant temperature (150 °C) and solvent-to-solid ratio (40 mL/g). [c] Extractives free. [d] Corrected for protein.

flow-through PLPW reactors, and yields are generally less than 10% of the total potential glucose available.[9,29] Vegas et al.[11] also noted the presence of significant amounts of gluco-oligosaccharides (GO) at mild processing conditions with only small

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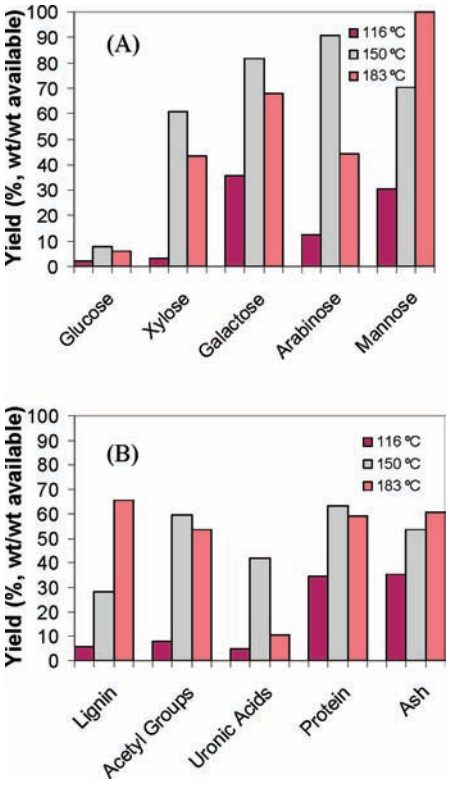

Figure 2. Effect of temperature on product yield obtained in freeze-dried liquid fractions at a constant flow rate of 150 mL/min and a solvent-to-solid ratio of 40 mL/g: (A) total carbohydrates (monomers and oligosaccharides) and (B) noncarbohydrate products.

increases in oligosaccharides production as the processing severity increased.

Hemicellulose. Hemicellulose in herbaceous plants consists of a main xylan chain of (1,4)-β-D-xylopyranosyl units with side chains of one to several α-L-arabinofuranosyl, β-D-galactopyranosyl, and β-D-glucuronopyranosyl units.[30] Because of its structure and branched nature, hemicellulose is amorphous and relatively susceptible to hydrolysis. In herbaceous plants, the most abundant carbohydrate in hemicellulose is xylan, with lesser amounts of arabinan, galactan, and mannan. Large amounts of xylan were hydrolyzed at temperatures of 150 °C and 183 °C, leaving a residue low in xylan content (Table 2). At 183 °C the residue contained only 2.6% xylan, which represents a theoretical xylan removal of nearly 94%. However, at 183 °C there was also considerable conversion of xylose to furfural and other degradation products. Overall, the greatest yield of xylose and xylo-oligosaccharides (total xylose) occurred at 150 °C, where 60% of the starting xylan in the native straw was obtained (Figure 2).

Total xylose content was greatest in the first two fractions at 183 °C, whereas there was a large amount of total xylose extracted in the first fraction at 150 °C, then a reduction, with a gradual increase as the course of the hydrolysis continued over the final fractions (Figure 3B). Initial high levels of hemicellulose hydrolysis and extraction probably occur with free xylan, which is not linked to the lignin in the biomass.[31] The delay in extraction of xylan may be related to the solubility behavior of xylo-oligosaccharides (XO) in solution. Solubility of XO with a degree of polymerization (DP) of six or less is not a limiting factor in uncatalyzed batch hydrolysis with PLPW, although for XO of higher DP solubility may be limiting.[32] Others have found that XO are not soluble until their DP has been reduced to less than 25.[11,31] Li et al.[14] found that there was a time lag in the presence of XO in the extracts of batch hydrothermal processing of xylan with PLPW and there was a shift in DP of XO with treatment time from higher DP to lower DP molecules. As high DP XO are released from the straw, they would need further hydrolysis to lower their DP enough to be solubilized and extracted from the reactor. The lag in xylan extraction may also be the result of structural changes and improved accessibility within the straw during the hydrolysis and extraction process. The disruption of lignin−carbohydrate complexes and the extraction of lignin from the biomass would make the previously shielded hemicellulose more accessible to hydrolysis.

The arabinose in herbaceous biomass can exist as furanosyl units attached as branched groups to some C-3 positions of the main xylan chain. This structure of arabinose makes it more susceptible to hydrolysis.[33] As a result, arabinose and arabino-oligosaccharide (total arabinose) yield from the straw was almost 90% (Figure 2), and it was hydrolyzed into monomeric form more easily than the other sugars (Figure 3). At 183 °C, there was a large loss of total arabinose from the liquid fractions compared to 150 °C, resulting in the production of furfural and other degradation products (Figure 3D).

Yields of total mannose (mannose and manno-oligosaccharides) and total galactose (galactose and galacto-oligosaccharides) in the liquid fractions was comparable to that of arabinose (Figure 2). In addition, mannan and galactan were easily hydrolyzed to monomers at higher temperatures (Figure 3C and 3E). At 183 °C, mannan was almost completely degraded to mannose monomers in the first two liquid fractions (Figure 3E) and close to 90% was extracted in monomer form overall (Table 2). Galacto-oligosaccharides (GOS) were fairly stable at temperatures below 183 °C and had comparable susceptibility to hydrolysis to monomers as arabinose. Mannan has a backbone of β-(1,4)- linked mannose units with short branches of one β-(1,6)-linked galactose unit. As with cellulose, the structure of mannan may form a compact configuration with many intermolecular hydrogen bonds, which often creates a crystalline formation that is difficult to solubilize.[34] However, the lower DP of mannin, when compared to cellulose, makes it more readily dissolved,[34] accounting for the larger yields when compared to those for cellulose.

Flow rate had little effect on the composition of carbohydrates in the liquid extracts (Table 2) or the carbohydrate content of individual fractions (Figure 4). There were only small reductions in the production of total galactose, total arabinose, and total mannose in the liquid extracts with an increase in flow rate. The greatest change in the extraction of carbohydrates occurred for the total xylose at the highest flow rate of 234 mL/min (Figure 4B). This reduction in the fractional content and yield due to flow rate could be attributed to mass transfer or limitations in the hydrolysis reactions of the system.

If an extraction is limited by the external mass transfer (solubility) of the solid to the solvent, then an increase in flow rate would result in an increase of the extraction rate of the solute. Conversely, if an extraction is limited by the internal mass transfer of the system (diffusion), then an increase in the flow rate would have little effect on the extraction of the solute. In these experiments flow rate does affect the extraction of xylose, but the result is a reduction in yield, not an increase, as would be expected if solubility were the limiting factor. In this case it is not a mass transfer problem, but one of reaction kinetics for the hydrolysis process. It is more probable that the reduction in xylan hydrolysis at 234 mL/min is due to the high flow rate, which would quickly remove any generated in situ acids from the

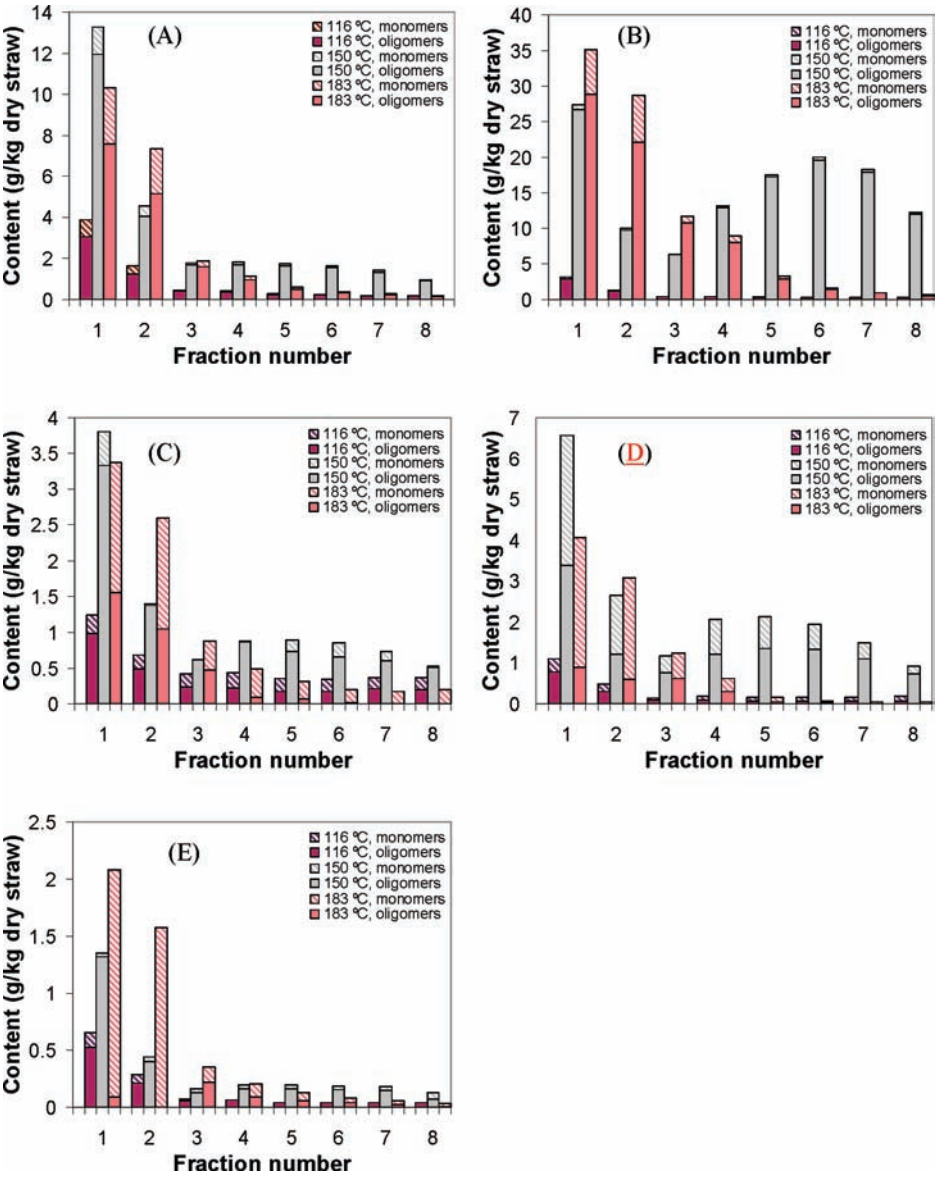

Figure 3. Effect of temperature on the total carbohydrate (monomers and oligosaccharides) content in freeze-dried liquid fractions collected for a solvent-to-solid ratio of 40 mL/g for triticale straw processed at a constant flow rate of 150 mL/min in PLPW [(A) glucose; (B) xylose; (C) galactose; (D) arabinose; (E) mannose].

reactor and reduce the residence time they are in contact with the biomass. This would in turn reduce the amount of xylan undergoing hydrolysis and thus lower the potential xylan extraction and yield from the reactor.

Acetyl Groups and Uronic Acids. There is a steady reduction of acetyl groups and uronic acids with an increase in temperature from the solid residues after hydrothermal treatment with PLPW of the triticale straw (Table 2). Yield of acetyl groups in the liquid extracts is 54−60% at 150 °C and 183 °C, respectively, and is approximately seven times greater than at 116 °C (Figure 2B). Uronic acid yield is only 4.8% at 116 °C, increasing to 42% at 150 °C, but only 10% at 183 °C (Figure 2B). The reduced accumulation of uronic acids at 183 °C is due to thermal degradation of the acid to 2-furancarboxylic and 5-formyl-2-furancarboxylic acids,[35] which were not measured. Formic and levulinic acids are produced from the degradation of furfural and HMF respectively.[36] Concentration of levulinic acid in the extracted dry matter remained low for all processing conditions. There is little difference in extraction of acetyl groups (1.29 and 1.27 g) and uronic acids (0.90 and 0.82 g) at 66 and 150 mL/min, respectively. Acetyl group production decreased by approximately 16% to 1.07 g, and uronic acid production decreased by nearly 25% to 0.64 g at a flow rate of 234 mL/min. This is corroborated by the small reduction in xylan hydrolysis at 234 mL/min. A reduction in xylan hydrolysis from reduced PLPW residence time in the reactor would result in fewer uronic monosaccharide or acetyl substituents being liberated from the biomass.

Lignin. Lignin content for the solid residues and liquid fractions has been corrected for protein. Protein can masquerade as lignin, producing a value that is higher than it should be.[20,37] All values were determined for native straw, and not the extractives free material specified by the NREL laboratory procedure,[20] because industry would use the material as received, which still contains the extractives. The extractives include nonstructural sugars, organic acids, inorganic material, nitrogenous material, chlorophyll, waxes, and other minor

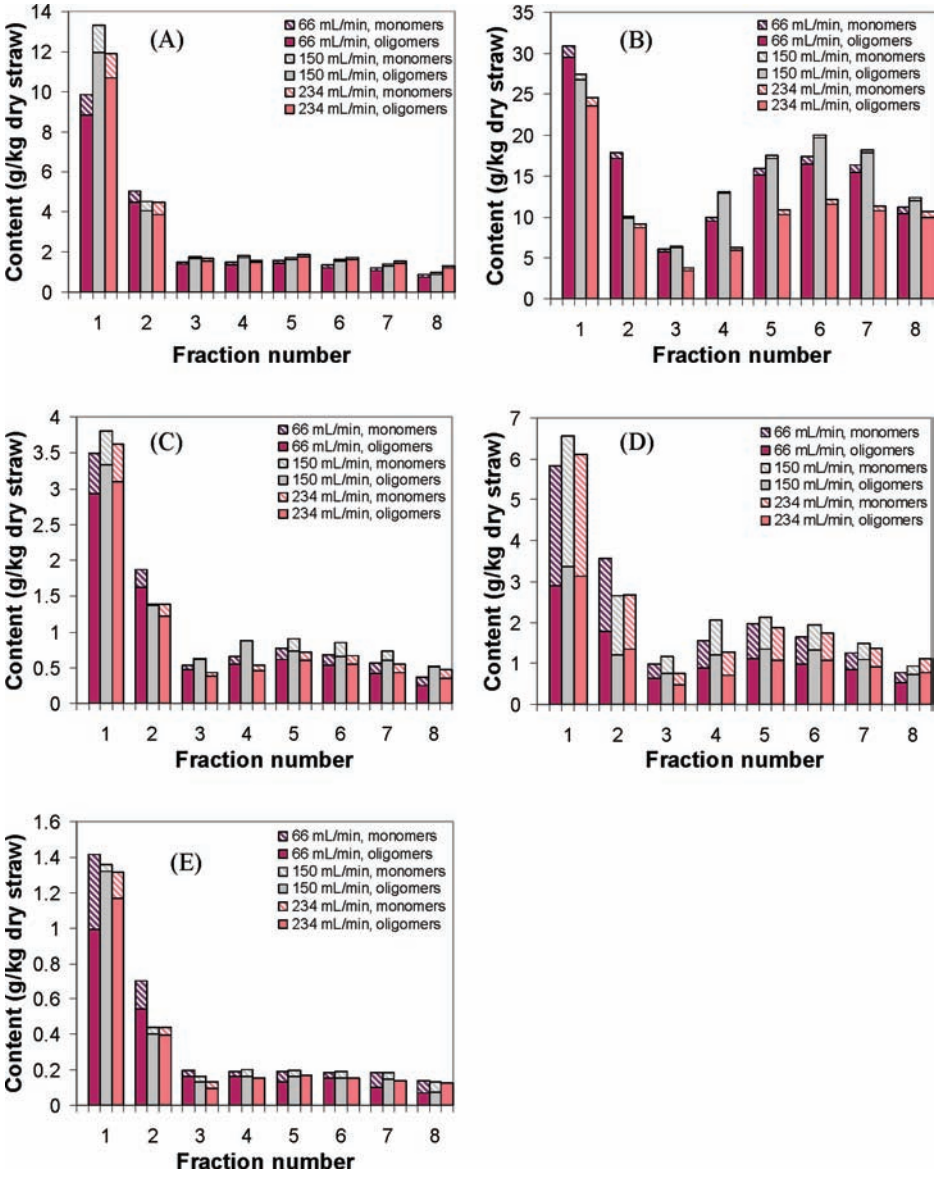

Figure 4. Effect of flow rate on total carbohydrate (monomers and oligosaccharides) content in freeze-dried liquid fractions collected for a solvent-to-solid ratio of 40 mL/g for triticale straw processed at 150 °C in PLPW [(A) glucose; (B) xylose; (C) galactose; (D) arabinose; (E) mannose].

components.[38] Extractive material in triticale straw can comprise 20% of the total mass and this material can have a significant effect on the measured lignin and ash contents, resulting in an overestimation of the lignin and ash content of the sample; however, there is no effect on the glucan and xylan content of triticale straw with the removal of the extractives.[5]

Increasing the processing temperature resulted in a greater portion of the lignin being removed from the triticale straw. At 116 °C, only 1.07 g of lignin was collected, increasing to 5.34 g at 150 °C, and 12.61 g at 183 °C, representing effective yields of 5.5, 27.5, and 64.9% of the starting lignin, respectively (Figure 2). The lignin yield in the extracts at 183 °C is overstated due to the presence of char-like substances, which remain in the residue after acid hydrolysis analysis of the samples for carbohydrate determination and cannot be separated from the remaining lignin. Similar yields using PLPW were achieved by other researchers for corn stover [10] and hardwood and grass species.[9]

In these experiments, the ratio of lignin to hemicellulose extraction was 0.60 at 116 °C and from 0.28−0.38 for all other conditions. Lignin does not exist in plant tissue as an independent polymer but is bonded with cellulose and hemicellulose, forming complexes with them.[3] In herbaceous crops, lignin is covalently bonded with carbohydrates (arabinoxylans) via ether- and ester-linked ferulic acids.[3] At 116 °C, there is minimal hydrolysis of the carbohydrates. Most of the material extracted is associated with the extractives contained in the native straw material. As such, lignin is not being liberated at this low temperature. At all other processing conditions, the lignin is being extracted in an almost linear fashion from the biomass along with the hemicellulose. Mok and Antal[9] theorized that water-solubilized lignin entered into solution with the hemicellulose as a complex oligomer and that this contributed to a linear correlation between hemicellulose recovery and the amount of lignin removed.

High PLPW flow rates were responsible for a 12% decrease in the amount of lignin extracted at 234 mL/min. Because of the lignin/phenolic−carbohydrate complexes formed in herbaceous

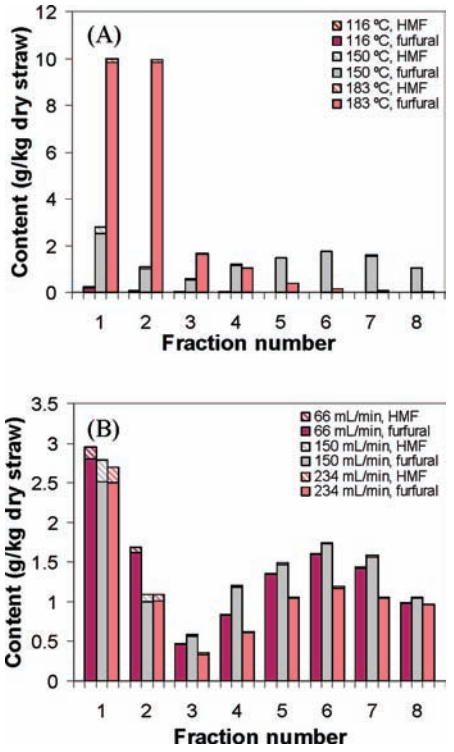

Figure 5. Effect of (A) temperature (flow rate of 150 mL/min) and (B) flow rate (temperature of 150 °C) on furan content in freeze-dried liquid fractions collected for a solvent-to-solid ratio of 40 mL/g for triticale straw processed in PLPW.

crops such as triticale, it is reasonable that with a reduction in the hydrolysis of hemicellulose, there would be a corresponding reduction in the extraction of lignin.

Protein and Ash. Concentrations of protein and ash in the liquid extracts were greatest in the earliest fractions and decreased as the process continued. The highest protein and ash concentrations occurred at 116 °C (Table 2); however, overall yields were lowest at 116 °C but still amounted to nearly 40% of the total amount available in the starting biomass (Figure 2B). Protein and ash are not bonded as strongly to the plant structure as hemicellulose and lignin. Therefore, they would be more easily extracted, even at mild processing temperatures. Material extracted at this temperature is most likely to be associated with the extractives contained in triticale straw.

Furans. Under severe hydrolysis conditions, the pentoses and hexoses in the biomass may be converted to the degradation products furfural and HMF, respectively, and are collectively known as furans. The production of furans during the hydrothermal treatment was small, and the concentration in the dried liquid extracts was low for processing temperatures of 116 °C and 150 °C (Table 2). However, at 183 °C, there was a large increase of both HMF and furfural in the extracts (Figure 5A). This is in agreement with the literature that shows production of furans occurring at temperatures above 170 °C.[39] Research has shown that the hexose monomers are fairly resistant to degradation to furans, with galactose as the most stable, followed by mannose and then glucose.[40] Thus, it is probable that HMF production was largely the result of the degradation of the glucose fraction. Kootstra et al.[36] found that during acid-catalyzed hydrolysis, xylose was more susceptible to degradation than arabinose. They also found that the degrading power of water alone was greater than fumaric and maleic acids but less than that of sulfuric acid. So, while the contribution of arabinose to furfural production is significant early in the extraction when a large portion of arabinose monomers were present, most furfural production was probably from the larger xylose fraction in the biomass.

There was little effect of flow rate on the production of furans (Figure 5B). There was a small decrease in furfural production at the highest flow rate, likely due to the reduced residence time of the extracts in the reactor. Production of furans in a flow-through reactor is usually low, especially when compared to batch systems because the hydrolysis products are constantly removed from the reactor.

Overall, hydrothermal treatment with PLPW yields a diverse set of monosacchardies, oligo-saccharides, lignins, and other compounds. The extraction of these bioproducts is dependent on the temperature of the process, but it is not greatly affected by the flow rate.

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