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Release of D-Xylose from Wheat Straw by Acid and Xylanase Hydrolysis and Purification of Xylitol

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Xylitol is a valuable sweetener produced from xylose-rich biomass. Our objective was to optimize conditions for maximum release of D-xylose from wheat straw by acid or enzyme hydrolysis with minimal release of other monosaccharides, and to purify xylitol from three other alditols. Ground straw was treated with 10 parts of 0.2–0.4 M sulfuric acid at 110–130 °C for 15–45 min or at reflux with 0.75–1.25 M sulfuric acid for 1.5–3 h. Under optimum conditions of either 0.3 M acid at 123 °C for 28 min or 1.0 M acid at 100 °C for 3 h, 18 or 19% of D-xylose plus ~ 6% other sugars were produced from straw (dry basis). A 16% yield of D-xylose plus 6% other sugars was obtained when hydrothermally (10% straw, 160 °C, 1 h) treated straw was incubated with a commercial xylanase. The lack of enzyme specificity for D-xylose release was attributed to the autohydrolysis of polysaccharides during the pretreatment plus slow hydrolysis of cellulose during enzyme digestion. Xylitol with a purity of 95% was obtained in 10% yield from straw after the reduction of an acid-hydrolyzate followed by fractional crystallization. Purification of the mixture of four alditols by open-column chromatography on a strongly basic anion-exchange resin in hydroxide form gave 7% xylitol crystals with a purity of 99%.

KEYWORDS: Wheat straw; hydrolysis; xylanase; D-xylose; xylitol

INTRODUCTION

Xylitol is a naturally occurring, five-carbon sugar alcohol with useful properties (*1, 2*). Xylitol's sweetness equals that of sucrose, but it is noncariogenic (*3*) and possibly even anticariogenic because it inhibits the growth of oral bacteria (*4*). Xylitol is passively absorbed from the digestive tract, independent of insulin and without elevation of blood glucose, which warrants its use in diabetic foods (*5*). Xylitol crystals possess a high negative heat of solution imparting a cooling sensation when ingested (*6*), an attribute that is pleasing in lozenges and chewing gum. The food energy value of xylitol is approximately 10.1 J/g (2.4 Cal/g) (*7*), which is approximately 40% below that of sucrose.

Xylitol is produced in three stages starting from wood or corncobs (*8*). Other potential xylan-rich starting materials include oat hulls, straw, coconut shells, almond shells, bagasse and cottonseed hulls, among others. The first step is swelling of fibers with simultaneous hydrolysis of the amorphous phase to release predominantly D-xylose. Swelling and hydrolysis typically are achieved by hot aqueous mineral acid. After cooling, the hydrolyzate is neutralized and the salts removed. In the second step, the neutralized hydrolyzate is hydrogenated over a nickel catalyst to produce a mixture of alditols. The third step is purification and isolation of xylitol, which appears to be achieved (*8, 9*) by differential complexation of alditols with metal ions (Ca^{2+}, Fe^{+++}, or Al^{+++}) held on strongly acidic cation-exchange resins (*10*). The chromatographic purification of xylitol from other alditols causes high production costs (*9*).

Recently, an electrochemical method (*11*) has been devised to produce xylitol in 50% yield by oxidative decarboxylation of sodium D-glucuronate to give the intermediate D-*xylo*penta-1, 5-diose, which is hydrogenated over Raney nickel to xylitol. Sodium D-glucuronate may be obtained by air-oxidation of methyl α-D-glucopyranoside. Microbial production of xylitol (*12, 13*) has been reported but not commercialized (*14*).

Approximately 2 billion bushels of wheat were produced in the United States in 2005, which translates to about 70 million metric tons of straw (*15*). Currently ~30% of wheat straw is used for fuel, cattle-feed, mulch, and bedding material for animals (*16*), and straw can be converted to pulp, paper, and fiberboard (*17*), and ethanol (*18*). Therefore wheat straw is increasingly being collected as a raw material. In addition, wheat straw contains nearly as much D-xylose in polysaccharide form as corncobs and wood (*19*). The production of xylitol offers a high-value use for wheat straw, and ~50% of the remaining solids would be available for ethanol production.

Reaction conditions that improve the selective release of D-xylose from wheat straw would reduce the production costs of xylitol, whether by acid- or enzyme-catalyzed hydrolysis. In

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addition, an improved chromatographic method to purify xylitol would be of benefit.

MATERIALS AND METHODS

Materials. Straw from a semi-dwarf, hard red winter wheat (*T. aestivum* var. Jagger) with a moisture content of 8% was ground in a Wiley mill (Model 4276) to pass through a 1-mm wire-mesh screen, and the ground straw was stored at room temperature in a sealed polyvinyl bag. Reference standards D-xylose, D-glucose, D-mannose, D-galactose, L-arabinose, D-fucose, xylitol, L-arabinitol, D-sorbitol, galactitol, *myo*-inositol, D-glucuronic acid, and furfural (all ≥ 99.0% purity) were obtained from Sigma-Aldrich (St. Louis, MO). Other chemicals were reagent-grade and from Fisher Scientific (Pittsburg, PA). Water for general use was distilled in a glass unit, whereas that used in high-performance anion-exchange chromatography (HPAEC) was purified to 18.3 ohms resistance with a Barnstead (D4700) NANOpure deionization system (Dubuque, IA). Ion-exchange resins, Dowex 2, Dowex 50W, Amberlite IRA-400, Amberlite IRA-120, Amberlite MB-150, and Amberlite IRA 743 were purchased from Sigma-Aldrich and Supelco (Bellenfonte, PA). Multifect Xylanase and SpezymeCP, derived from genetically modified *Trichoderma reesei*, were kindly donated by Genencor International (Palo Alto, CA), while Natugrain Wheat L was donated by BASF (Shreveport, LA). Xylanase M6 from a rumen microorganism was acquired from Megazyme International (Bray, Ireland), and a xylanase from *Thermomyces lanuginosus*, which is a product of Novozyme (Franklinton, NC), was acquired from Sigma-Aldrich.

Endo-1, 4-β-xylanase activities were determined on an insoluble substrate (Xylazyme AX tablet, Megazyme) at the optimal pH and temperature as indicated by each manufacturer. Endoxylanase activity was calculated relative to a xylanase standard of known activity (IU) in the kit. One international unit (IU) of endoxylanase activity in the reference standard is that required to liberate 1.0 micromole of reducing end (measured as D-xylose equivalents) per minute from 0.5% arabinoxylan solution at pH 3.5 and 40 °C. β-xylosidase activity was determined on the basis of the Sigma-Aldrich protocol with the substrate *O*-nitrophenyl β-D-xylopyranoside (Sigma-Aldrich). One IU of β-xylosidase activity is the enzyme activity required to hydrolyze 1.0 micromole of *O*-nitrophenyl β-D-xyloside to *O*-nitrophenol and D-xylose per minute at pH 5.0 and 25 °C.

Wheat Straw Composition. Moisture, protein, lipid, and ash contents were determined by Approved Methods of AACC International (*20*). The polysaccharides in straw were hydrolyzed according to Browning (*21*), and the monosaccharide composition determined. Ground wheat straw (1.0g) was mixed with 72% sulfuric acid (10 mL), and the mixture held at 30 °C for 1 h with stirring. The concentration of acid in the mixture was adjusted to 4.0% by adding water (170 mL), and the mixture heated at reflux for 2 h. An aliquot (20 μL) of the hydrolyzate was made to volume (10 mL) with water containing an internal standard, and the sugars were assayed as described below. Uronic acid in straw was determined on the basis of the method of Blumenkratz and Asboe–Hansen (*22*), and acetyl content was estimated by saponification (*23*). Klason lignin content of straw was measured using the gravimetric method reported by Lawther et al. (*24*), except that the hydrolysis of straw was accomplished at 30 °C for 1 h in 72% sulfuric acid, followed by boiling in 4.0% sulfuric acid for 2 h. All tests were carried out in duplicate or triplicate and reported on a dry-weight basis.

Analysis of Sugars and Sugar Alcohols. Sugars and sugar alcohols were assayed by high-performance anion-exchange chromatography (HPAEC) on a liquid chromatography system comprising a high-pressure GD 50 gradient pump and a pulsed amperometric detector (PAD), both from Dionex (Sunnyvale, CA), a Rheodyne (Rheodyne LLC, Rohnert, CA) injector with a 20 μL loop, and a Shimadzu Chromatopac integrator, Model CR601 (Kyoto, Japan). Pulse potentials (volts) and durations (sec) on the PAD were as follows: $E_1 = +0.05$ ($t_1 = 0$), $E_2 = +0.05$ ($t_2 = 0.2$), $E_3 = +0.05$ ($t_3 = 0.4$), $E_4 = +0.75$ ($t_4 = 0.41$), $E_5 = +0.75$ ($t_5 = 0.6$), $E_6 = +0.15$ ($t_6 = 0.61$), $E_7 = -0.15$ ($t_7 = 1$). Elution of sugars (*25*) was done isocratically using 20 mM sodium hydroxide at a flow rate of 1.0 mL/min on a Dionex Carbopac PA1 (4 × 250 mm) column fitted with a guard column (Carbopac PA2 4 × 50 mm). Elution of sugar alcohols (*25*) was done isocratically using 0.6 M sodium hydroxide at a flow rate of 0.4 mL/min on a Carbopac MA1 column. Internal standards, D-fucose and *myo*-inositol, were injected with the samples for the quantification of sugars and sugar alcohols, respectively. All samples were passed through a 0.45 μm membrane filter before injection, and analyses were carried out in duplicate at ambient temperature. The quantities of sugars and sugar alcohols injected into the HPAEC-PAD system varied between 100 and 200 ng. The PAD detection limit for a sugar was ~40 ng, which translates to ~0.04% of straw weight.

Furfural Determination. Furfural and hydroxymethyl furfural were analyzed together by absorbance at 277 nm using a standard curve derived from known levels of furfural in water (*26*).

Partial Acid-Catalyzed Hydrolysis of Wheat Straw. Pressurized hydrolysis experiments were carried out in a 1 L Parr reactor (Model 4521, Parr Instrument Co., Molin, IL). Ground wheat straw (10 g) was mixed with dilute sulfuric acid (100 mL) in the reactor vessel. The reactor was sealed and the contents stirred at a moderate rate. The reactor was heated to temperature over an average time of 11 min, and time zero was taken when the desired temperature was reached. In a modeling experiment, the independent variables and their levels were as follows: acid concentration, 0.2, 0.3, and 0.4 M; temperature, 110, 120, and 130 °C; and reaction time, 15, 30, and 45 min. The dependent variables were the yield of D-xylose and the selectivity expressed as the molar ratio of D-xylose to other monosaccharides. Absolute pressure in the reactor was calculated as saturated steam pressure at each temperature and equaled 446.1 to 711.5 kPa (4.4–7.0 atm). At atmospheric pressure, the straw (1 part) was hydrolyzed by heating at reflux in 0.75, 1.0, and 1.5 M sulfuric acid (10 parts) for 1.5, 2, 2.5, and 3 h. At the end of a reaction, the hydrolyzate was cooled quickly using the cooling coil of the reactor. The liquid phase was recovered after filtration and analyzed for sugars. Yields (%) of monosaccharides were calculated using the formula: $Y = (c)(d)(v)(100)/M$, where c is the concentration of a sugar (g/L), d is the dilution factor, v is the volume of the liquid phase hydrolyzate (L), and M is the mass of dry wheat straw (g).

Hydrothermal Pretreatment of Wheat Straw. Ground wheat straw (10.0 g) and water (100 mL) were charged into the Parr reactor. The reactor was sealed, the stirrer started, and the temperature was brought to 140, 160, 180, or 200 °C within 15 min (0.88–3.20 MPa, 9–32 atm), which was recorded as time zero. The charge was cooled and the liquid phase and the straw residue were recovered by filtration through Whatman 1 filter paper. The solid phase was washed several times with hot water, and the swollen residue held in a freezer (−15 °C) for less than two months prior to assay. The washings were combined with the liquid phase and the composite made to 0.02% sodium azide.

Monosaccharides released into the liquid phase during hydrothermal treatment of straw were determined by HPAEC-PAD. To determine the total level, free plus combined, of a monosaccharide, an aliquot of the liquid phase (~1.0 mL) from hydrothermally treated wheat straw was made to 4.0% sulfuric acid (0.7 M), and the mixture heated at reflux for 2 h before sugar analysis. Sugars in oligomeric form were estimated by subtracting the free monosaccharide contents in the liquid phase from the total level. The presence of xylooligosacchardes with chain length ≤ 6 in the liquid phase from pretreated straw was verified by chromatography of xylooligosaccharides obtained from Megazyme International.

Enzymatic Hydrolysis of the Solid Phase Isolated from Hydrothermally Treated Straw. Wet, hydrothermally treated straw residue (4.0 g), with an average moisture content of 85%, was mixed with 25 mM acetate buffer (pH 4.7, ~40 mL) containing 0.02% sodium azide and Multifect xylanase to form a 1.5% straw (ds) slurry with xylanase activity of 20 IU per gram of dry straw. The mixture was incubated in a reciprocal-shaking water bath (120 rpm) (Precision Scientific Model 9506-204) at 45 °C for 24 h. Aliquots were drawn with time, and ethanol was added to a concentration of 70% to quench the reaction. Other xylanases obtained from BASF, Megazyme, Novozyme, and a cellulase from Genencor also were tested at 20 IUg^{-1}dry straw.

Reduction of Wheat Straw Hydrolyzate and Fractional Crystallization of Xylitol. A sample (10.0 g) of ground wheat straw was

hydrolyzed with 0.3 M sulfuric acid (100 mL) at 123 °C for 30 min in the Parr reactor. The slightly yellow-colored liquid phase of the hydrolyzate was recovered by filtration, neutralized with 0.5 M sodium hydroxide, and the neutralized solution stirred for 1.5 h at 45 °C with activated charcoal (15 g). The charcoal was removed by filtering through a glass microfiber filter pad (cat. no. C170-500, Fisher Scientific), and the clear solution concentrated to ~10 mL under reduced pressure at 50 °C. The concentrate deposited crystals of sodium sulfate, which were collected by filtering and were washed twice with 80% ethanol (~50 mL) to give 2.0 g, 48% yield. The washings and filtrate were combined and the level of D-xylose determined. The solution was stirred with 150 mL Amberlite MB-150 mixed-bed resin (H^+ and OH^-) for 1.5 h and the resin removed by filtration. The filtrate, which was assayed for monosaccharides present, was then concentrated under vacuum to ~10 mL, and the concentrate added dropwise to 10 mL of 20 mM sodium borohydride in water. After stirring for 2 h at 25 °C, the reaction was quenched and sodium ions removed by stirring the solution with 150 mL of Amberlite IRA-120 (H^+) resin for 1 h. After removing the resin, boric acid was removed from the filtrate by its passage through a column of Amberlite IRA-743 (200 mL). The effluent was concentrated under vacuum at 50 °C to a syrup and 90% ethanol added to the syrup. After 48 h at 2 °C, the xylitol crystals were collected, dried, and weighed. The mother liquors were reprocessed to obtain a second batch of crystals. The two batches of crystals amounted to 0.75 g and 0.31 g, respectively. Analysis of the combined crystals by HPAEC-PAD showed the presence of 95% xylitol, 3% L-arabinitol, and 2% D-sorbitol.

Purification of Xylitol by Open-Column Ion-Exchange Chromatography. A glass column with an internal diameter of 2.5 cm and a length of 50 cm was loaded with Dowex 2 resin (100–200 mesh) in the chloride form (~120 g wet weight). The resin was converted to the hydroxide form in the normal manner, and a portion (0.25 g) of the reduced wheat straw hydrolyzate (total of 2.1 g from 10 g of straw) was added to the top of the column. The column was developed with water at a flow rate of 5 mL/min, and 10 mL fractions were collected. The fractions were assayed for alditols by HPAEC-PAD, and fractions 16–21 (xylitol) and fractions 22–29 (xylitol rich) were combined. Fractions 30–44 containing mostly D-sorbitol with some L-arabinitol and galactitol were discarded. The separation of the reduced hydrolyzate was repeated (7 × 0.25 g) to give xylitol plus xylitol-rich fractions, and the latter were combined and rechromatographed. All xylitol fractions were combined and concentrated under vacuum to produce a solid (0.7 g, 7%) that was recrystallized from 50% ethanol. After cooling overnight at 5 °C, the xylitol crystals (0.5 g) were found to contain less than 1% of L-arabinitol and D-sorbitol, and they melted at 90–92 °C; literature (*27*), m.p. 93–94.5 °C.

Statistical Analysis. For acid hydrolysis at 110–130 °C, the Box-Behnken experimental design was used with two replicates at the center point giving a total of 14 runs, all randomized. For acid hydrolysis at 100 °C a single replicate, full-factorial design with two extra center points, and a total number of 14 randomized runs were done. A quadratic polynomic model was used to calculate the response surface.

RESULTS AND DISCUSSION

Composition of Jagger Wheat Straw. Jagger wheat straw was hand-harvested and was free of leaves and debris; **Table 1** shows the proportions of sugars in the straw. D-Glucose and D-xylose together comprised over three-fourths of the sugars in Jagger straw with D-glucose present at almost twice the concentration of D-xylose (42 vs 22% of straw). Whereas lignocellulosic materials contain more cellulose than hemicellulose, the hemicellulose fraction is more accessible to hydrolysis than cellulose. Hemicellulose occurs mostly as amorphous xyloglucan in type I plant cell walls or as amorphous glucuronoarabinoxylan in type II walls in the grass family (*28*).

The D-xylose content of straw, which was corrected for losses during hot-acid hydrolysis, was comparable (22–23%) between laboratories except for the 19% reported by Chesson (*29*). The combined levels of L-arabinose, D-galactose and D-mannose amounted to 3.6–4.2% of wheat straw (*30, 31*). Hexuronic acid in straw was found to be 3.3%, most of which is probably D-glucuronate linked to the xylan chain (*32*), but some may be D-galacturonate from pectic substances (*22*). Acetyl content of the straw was estimated to be 1.3%, within the range of 1–2% reported by Bacon (*33*).

Table 1. Sugars in Wheat Straw

	percent of dry straw			
		unknown variety		
sugar	Jagger[a]	Harper and Lynch (*30*)	Chesson et al. (*29*)	Jacobs (*31*)
D-glucose	41.7	36.4	36.0	38.3
D-xylose	22.0	23.4	18.8	22.4
L-arabinose	2.7	3.8	2.7	2.9
D-galactose	0.9		0.8	0.7
D-mannose			0.7	0.4

[a] Values for Jagger wheat straw were determined in triplicate and were corrected for losses of sugars during hydrolysis in boiling 0.7 M sulfuric acid for 2 h. The coefficient of variation of the sugar percentages was less than 5%.

Table 2. Composition of Jagger Wheat Straw

component	percent dry basis[a]
carbohydrates (67.9 × 0.9)	61.1 ± 0.8
klason lignin	20.1 ± 1.0
protein	4.8 ± 0.5
lipid	0.5 ± 0.1
hexuronic acid	3.3 ± 0.2
ash total	8.4 ± 0.2
acetyl	1.3 ± 0.1
total	**99.5**

The Klason lignin content of Jagger wheat straw was about 20%, intermediate between the 14–25% lignin (*24, 30, 34*) reported before. Klason lignin tends to be elevated because it includes polyphenols, cutin, and some nitrogenous matter (*35*). Protein and ash accounted for 4.4% and 8.4%, respectively, of Jagger straw (**Table 2**). The protein was within the range of 2.4 to 5.8% given by Theander and Åman (*35*), but the ash content of 8.4% was higher than the 4–6% of Harper and Lynch (*30*). Straw from the semi-dwarf Jagger wheat was found to contain a considerable level of silicon dioxide.

Optimum Release of D-Xylose from Straw by Acid-Catalyzed Hydrolysis at Elevated Pressure. Response surface methodology was applied to the acid-catalyzed, pressure hydrolysis of Jagger straw to predict the highest yield of D-xylose with highest selectivity. The second-order model predicted a maximum yield of 19% D-xylose (straw basis) at an acid concentration of 0.3 M, temperature of 123 °C (pressure = 4.9 atm) and a duration of 28 min, which excluded the 11 min preheating time. **Figure 1** shows the yield response at 30 min for straw in 0.2–0.4 M sulfuric acid at 110–130 °C. The predicted yield of 19% was confirmed experimentally and represented a recovery of 85% of the 22% D-xylose in the straw. UV analysis of the optimum hydrolyzate indicated that 1% D-xylose may have been dehydrated to furfural, while the other 2% was lost in the insoluble residue or caramelized.

During acid-catalyzed hydrolysis of the straw to produce D-xylose, three other monosaccharides were released, L-arabinose, D-glucose, and some D-galactose; no D-mannose nor D-gluco- or D-galacturonic acids were detected. Because a mixture of sugars was released, the other response modeled in the acid-hydrolysis reaction was selectivity. Under the conditions giving maximum yield of D-xylose at 123 °C in 0.3 M sulfuric

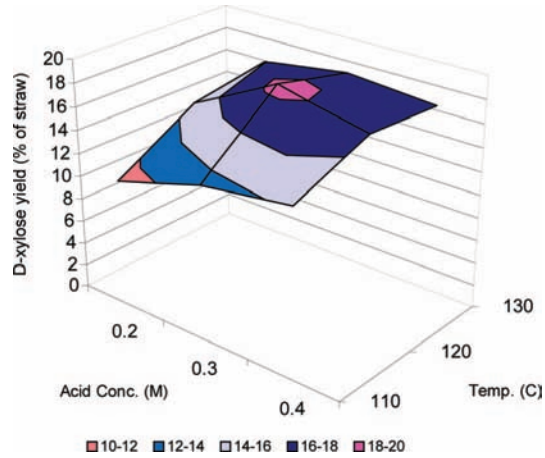

Figure 1. Yield of D-xylose after 30 min of pressure hydrolysis of ground wheat straw in 10 parts of 0.2–0.4 M sulfuric acid at 110–130 °C. The regression equation with linear and quadratic terms gave $R = 0.85$ ($p < 0.05$).

acid for 30 min, the model for selectivity (data not shown) gave a value of 3.3 mol D-xylose/mol of other monosaccharides. Selectivity remained above 3 for most hydrolysis conditions except when conditions became harsh, that is, a combination above 120 °C and above 0.3 M sulfuric acid. The decrease in selectivity at harsh hydrolysis conditions resulted from two changes, a decrease in the D-xylose level because of sugar dehydration and an increase in the D-glucose level due to accelerated hydrolysis of cellulose. A selectivity above 3 under mild hydrolysis conditions is of little consequence because the yields of D-xylose are too low.

In the present work, D-glucose released from straw at 110–130 °C in 0.2–0.4 M sulfuric acid for 30 min ranged from 0.8%–2.6% based on dry wheat straw or ~2–6% based on its cellulose content, assuming all D-glucose in straw occurs as cellulose. Under optimum pressure hydrolysis (123 °C, 0.3 M sulfuric acid, 30 min), the level of D-glucose in the hydrolyzate was 2.4% of that in dry straw. Cellulose hydrolysis usually does not exceed 10–17% when lignocellulosics are subjected to hot dilute mineral acids that causes a maximum release of D-xylose (*36*). The ever increasing level of D-glucose as hydrolysis proceeds shows that more cellulose is being exposed as the xylan is removed.

The level of L-arabinose released under the optimum pressure-hydrolysis conditions was 1.9% of straw (70% recovery), whereas the level of D-galactose was 0.5% (50% recovery). It is well known that the furanose ring of L-arabinose that is attached to the xylan backbone is hydrolyzed rapidly compared to the pyranose ring of the other sugars (*37*).

Optimum Release of D-Xylose from Straw by Acid-Catalyzed Hydrolysis at Atmospheric Pressure. Figure 2 shows the yield of D-xylose when ground straw was heated in boiling 0.75–1.25 M sulfuric acid for 1.5–3 h at atmospheric pressure. The yield of D-xylose ranged from 13 to 18% with the highest obtained in 1.0 M acid for 3 h or 1.25 M acid for 2.5 h.

The optimum yields of D-xylose were nearly the same (18 vs 19%) for acid-catalyzed hydrolysis under atmospheric and elevated pressure. However, hydrolysis at atmospheric pressure required about three times more acid and six times longer, which agrees with data on sugar cane bagasse hydrolysis (*38*).

Hydrothermal Pretreatment of Wheat Straw Before Enzymolysis: Carbohydrates Released in the Liquid Phase. Lignocellulosic biomass must be pretreated to increase enzyme

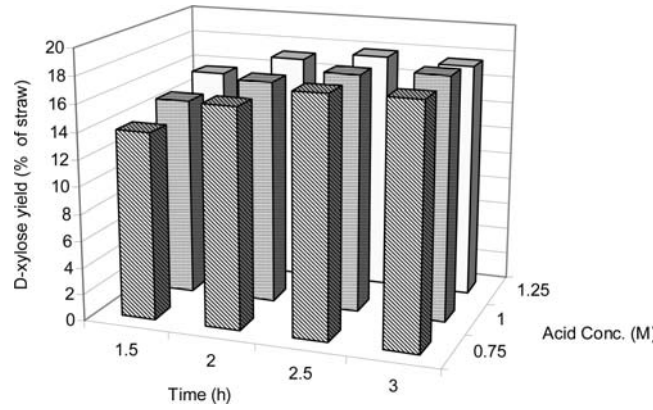

Figure 2. Yield of D-xylose from ground wheat straw heated in 10 parts of boiling 0.75–1.25 M sulfuric acid for 1.5–3.0 h.

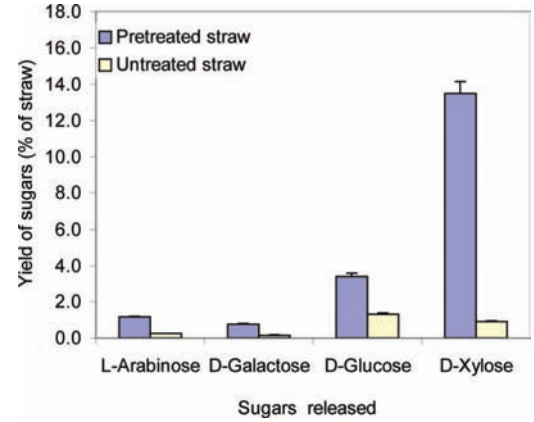

Figure 3. Monosaccharides (coefficient of variance 5.0%) released by *T. reesei* (Multifect ®) xylanase (40 IU/g dry straw) after incubation of untreated and hydrothermally treated wheat straw (10% straw, 160 °C, 1 h). The digestion was done on 2.5% straw at pH 4.7 and 45 °C for 12 h.

accessibility to its polysaccharides (*39*). One such pretreatment is heating biomass under pressure in water followed by reducing the pressure and cooling, which causes swelling and opening of the fibrous structure. Hydrothermal treatment also is referred to as autohydrolysis (*40*) because of acidity generated by the uronic acids and by the hydrolytic release of acetic acid (*41*), which then catalyzes the formation of additional organic acids. The hot acidic solution causes partial hydrolysis of the polysaccharides (*42*).

To determine the best conditions for hydrothermal treatment, ground wheat straw at 10% in water was heated at temperatures of 140, 160, 180, and 200 °C for 0.25, 0.5, and 1 h. The benefit of hydrothermal treatment to xylan hydrolysis is illustrated in **Figure 3**. Hydrothermal pretreatment increased the yield of D-xylose almost 16 times above that found for ground untreated straw. At the same time, the pretreatment increased the yields of L-arabinose, D-galactose, and D-glucose by 1.1%, 0.7%, and 2.4%, respectively. When the straw was chopped into small pieces of about 1 cm length and subjected to the same hydrothermal pretreatment followed by xylanase hydrolysis, the yield of D-xylose was 6% lower than that for ground straw.

Figure 4 outlines the hydrothermal treatment of ground wheat straw, the separation of a treated mixture into its liquid and solid phases, and the various hydrolytic treatments and carbohydrate assays. Sugar analyses of the solid and liquid phases of the hydrothermally treated straw was done to determine if enzyme–inhibitory compounds (*43*) might have been released in the liquid phase during the hydrothermal treatment of straw.

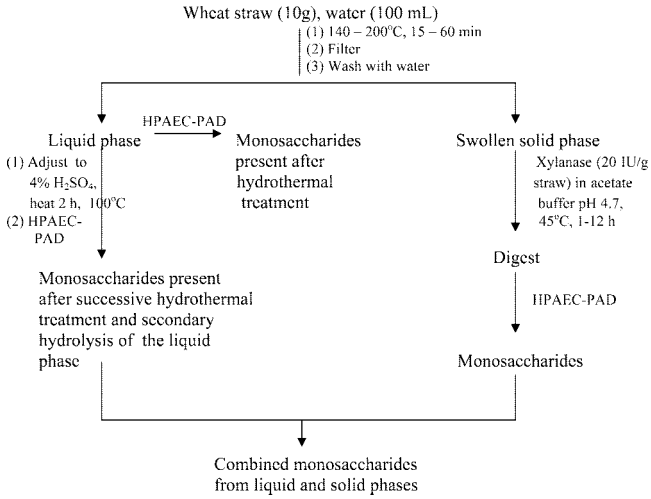

Figure 4. Hydrothermal treatment of ground wheat straw and subsequent hydrolysis and sugar assays of the liquid and solid phases.

Table 3. Total Sugars (Free and Combined Forms) (% of Dry Straw) and Free Sugars in the Liquid Phase after Hydrothermal Treatment of Straw at Different Time–Temperature Combinations

		total and free sugar[a]			
temp (°C)/time (min)		L-arabinose	D-galactose	D-glucose	D-xylose
140					
	15	0.4 (0.1)	0.2 (nd)[b]	0.4 (0.1)	0.4 (nd)
	30	0.4 (0.2)	0.2 (nd)	0.6 (0.2)	0.5 (nd)
	60	1.0 (0.3)	0.3 (nd)	0.9 (0.2)	1.0 (nd)
160					
	15	1.3 (0.4)	0.5 (nd)	1.0 (0.1)	1.8 (0.1)
	30	1.6 (0.5)	0.4 (nd)	1.0 (0.1)	2.9 (0.1)
	60	2.1 (0.8)	0.7 (0.1)	1.4 (0.1)	8.0 (0.1)
180					
	15	1.6 (0.6)	0.6 (0.1)	1.6 (0.1)	9.3 (0.2)
	30	1.3 (0.6)	0.8 (0.1)	0.5 (0.1)	10.6 (1.4)
	60	0.6 (0.4)	0.5 (0.3)	1.3 (0.2)	9.0 (2.0)
200					
	15	0.3 (0.2)	0.4 (0.2)	1.6 (0.2)	7.5 (2.2)
	30	0.1 (0.1)	0.3 (0.2)	1.5 (0.3)	1.6 (1.0)
	60	nd	nd	0.6 (0.2)	0.2 (0.1)

[a] The total sugar is the sum of free and combined forms. The free sugar is given in parentheses. [b] Not detected. Sugars were lost in hot boiling 0.7 M sulfuric acid for 2 h.

Sugars determined by acid hydrolysis of the liquid phase, added together with those determined by enzyme hydrolysis of the solid phase, indicated the highest potential yield of D-xylose that might be obtained by xylanase hydrolysis. In other experiments, as illustrated in **Figure 3**, the two phases were not separated but were hydrolyzed together by added xylanase. All hydrothermal treatments of ground straw in the Parr reactor were done in 10 parts of water. Heating straw with less than about 10 parts of water often caused charring of the straw because the slurry was immobile.

Table 3 shows the concentrations of the total (free and combined forms) and the free monosaccharides in the liquid phase after the hydrothermal treatment of wheat straw. The extent of solubilization of carbohydrates from ground straw increased and then decreased with increasing temperature/duration of hydrothermal treatment. At 140 °C, there was limited sugar (<1% of straw) in free and oligomeric forms present in the liquid phase. At 160 °C, the total level of all saccharides increased, especially that (8.0% of straw weight) of D-xylose released in 60 min (**Table 3**).

The level of total D-xylose in the liquid phase continued to increase up to 10.6% at 180 °C pretreatment for 30 min, whereas the total levels of the other saccharides released did not increase or they decreased (**Table 3**). At 200 °C pretreatment, the levels of the free and combined forms of all solubilized saccharides decreased below those observed at 160 °C. High temperature and/or prolonged heating resulted in a loss of D-xylose, probably because it was converted to furfural (*26*), or it reacted with peptide amino groups to give Maillard browning products that resulted in a dark color and a burnt-sugar odor.

Even though hydrothermal treatment of straw at 180 °C for 30 min gave the highest release of total D-xylose (10.6%) in the liquid phase, the solid phase obtained after that pretreatment released less xylose (~3%) upon xylanase hydrolysis than after pretreatment at 160 °C for 1 h (~7%) (see below). The optimum temperature and time for hydrothermal treatment of the ground straw, judging by the total yield of D-xylose, was 160 °C for 1 h.

The pH of the liquid phase after the hydrothermal treatment of straw varied from pH 5 at 140 °C to pH 3 at 200 °C, both after 60 min of heating. At 160 °C and 1 h, which was the optimum pretreatment, the acidity generated was equivalent to 2.5 mM as determined by titration with alkali. Assuming the acidity was generated by the release of acetic acid, the value was equivalent to ~1% acetyl based on straw weight, which was slightly lower than the experimental value of 1.3% (**Table 2**) and within the 1–2% acetyl content of wheat straw reported by Bacon et al. (*23*).

The chromatography data in **Table 3** shows that after hydrothermal treatment of straw at 160 and 180 °C, the liquid phase contained mainly oligosaccharides rather than free sugars. After pretreatment of 160 °C for 60 min or 180 °C for 15–60 min, the oligosaccharides in the liquid phase accounted for 77–98% of the D-xylose in that phase. Xia et al. (*44*) subjected oat hulls to autohydrolysis at 180 and 200 °C for 30 min and reported that 50% of xylan was released into the hydrolysis liquor in oligomeric form with dp > 10, whereas Nabarlatz et al. (*45*) autohydrolyzed wheat straw and corn cobs at 179 °C for 23 min and obtained 41 and 59%, respectively, of xylooligosaccharides based on xylan content. Hydrothermal treatment of wood with (*46*) or without (*40*) acetic acid also released 50% of the xylan in the form of xylooligosaccharides.

Enzymatic Release of Monosaccharides from the Solid Phase of Hydrothermally Treated Wheat Straw. After hydrothermal treatment of straw at 160 °C for 1 h, the swollen solid phase was isolated (**Figure 4**) and incubated with the commercial xylanases at their optimum temperatures and pH values (**Table 4**). In those enzymolysis experiments, endoxylanase concentration was maintained at 20 IUg^{-1}dry straw; an increase in enzyme concentration to 40 IUg^{-1} did not increase the release of D-xylose (data not given). **Figure 5** shows that Nutagrain Wheat L (BASF) yielded 1.8% of free D-xylose, Xylanase M6 (Megazyme) 1.2%, and X2753-50G (Novozyme) 0.2% based on dry straw weight. However, Multifect (Genencor) released 9% free D-xylose based on straw. The cellulase (Spezyme CP from Genencor) also showed xylanase activity (**Table 4**) and produced about as much D-xylose as the Multifect xylanase, but Spezyme CP released much more D-glucose (Fig **5**). The solid phase isolated after hydrothermal treatment at 160 °C for 1 h was calculated from the data in **Tables 1** and **3** to contain 14% D-xylose based on straw; therefore, the Multifect enzyme released about two-thirds of the D-xylose remaining in the residue.

In addition to possessing high endo-xylanase activity on insoluble xylan, Multifect xylanase also contained the highest

Table 4. Endo-xylanase and Beta-xylosidase Activities of Commercial Enzymes[a]

source	commercial name	optimum activity[b] pH	optimum activity[b] temp >(°C)	endo-xylanase[c] activity (IU/mL)	beta-xylosidase[d] activity (IU/mL)
Xylanases					
Genencor	Multifect	5	55	984	44
Novozyme	X2753–50G	5	75	1356[e]	24
Megazyme	Xylanase M6	3	40	3	2
BASF	Natugrain wheat L	4	40	2936	2
Cellulase					
Genencor	Spezyme CP[f]	5	55	432	18

[a] All enzymes were obtained in the dissolved state except for Novozyme X2753-50G. [b] Optimum pH and temperature reported by manufacturer. [c] Activity determined using Megazyme endo-xylanase diagnostic kit. [d] Activity determined using Sigma beta-xylosidase activity protocol. [e] Activity reported in IU/g of solid enzyme. [f] Enzyme preparation sold as cellulase.

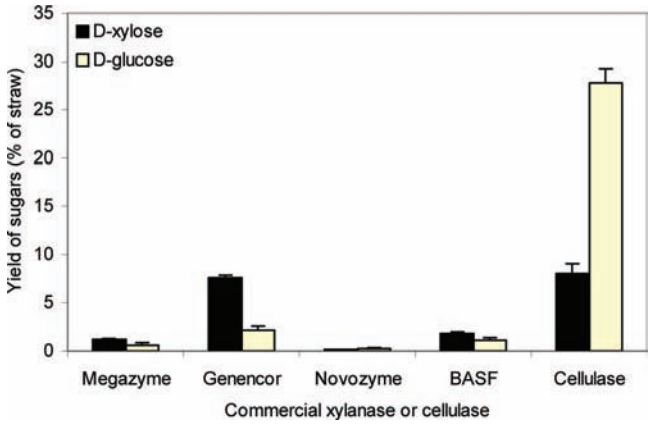

Figure 5. Yields of D-xylose and D-glucose produced by enzyme hydrolysis of the swollen solid phase from wheat straw hydrothermally treated at ~10% solids for 1 h at 160 °C. The swollen solid phase was incubated at 1.5% dry solids with a cellulase or with various xylanases at a 20 IU/g dry straw and at optimum pH and temperature (**Table 4**) for 12 h.

beta-xylosidase activity on a soluble xyloside (**Table 4**), which may explain its efficiency in releasing D-xylose from xylan. On the contrary, even though the BASF enzyme had the highest endo-xylanase activity, it had the lowest beta-xylosidase activity corresponding to its ineffectiveness in yielding D-xylose. Megazyme xylanase also has low β-xylosidase activity and low endoxylanase activity on insoluble xylan, and it produced <2% D-xylose from the swollen solid phase (**Figure 5**). Poutanen and Puls (*47*) also found that D-xylose released by the xylanases was better correlated to beta-xylosidase rather than endo-xylanase activity. Novozyme xylanase showed appreciable levels of both endo-xylanase and beta-xylosidase activity (**Table 4**) but yielded the least amount of D-xylose (Fig **5**) from the swollen solid phase, which remains unexplained.

The yields of D-xylose and D-glucose released by Multifect xylanase hydrolysis of the swollen solid phase from the 160 °C pretreatment for 15, 30, and 60 min are shown in **Figures 6A** and **6B**, respectively. The release of D-xylose after 12 h xylanase digestion of the solid phase was the highest at 8% of straw when the pretreatment at 160 °C lasted 30 min (**Figure 6A**). Beyond the 12 h incubation with xylanase, there was essentially no increase in D-xylose yield (data not shown). Release of D-glucose from the swollen solid phase amounted to 1% of straw weight after incubation for 12 h (Fig **6B**). Little or no L-arabinose or D-galactose was detected in the enzyme digests of the solid swollen phase because most of those sugars had been released into the liquid phase.

When straw was hydrothermally treated at 180 °C, Multifect digestion of the swollen solid phase yielded less D-xylose as pretreatment time increased from 15 to 60 min (**Figure 6C**), whereas the level of D-glucose released remained constant at ~1% (**Figure 6D**). At 140 °C pretreatment (data not shown), a yield of only 4–5% D-xylose was obtained upon digestion of the swollen residue, which was attributed to the limited swelling of the fibrous tissue. At 200 °C pretreatment, Multifect digestion of the swollen residue gave only ~1% D-xylose (data not shown). The dark color and caramel odor of the 200 °C pretreated straw indicated that most of the D-xylose had been released into the liquid phase where it was degraded or otherwise coupled to other reactants in the hot acidic environment.

Potential D-Xylose Yield by Xylanase Hydrolysis of Hydrothermally Treated Wheat Straw. For each of the 12 hydrothermal treatments, the total (free and oligomeric) D-xylose in the liquid phase (**Table 3**) was added to the D-xylose released by xylanase hydrolysis of the solid phase to give the combined yield of D-xylose. **Figure 7** shows that the highest potential combined yield of D-xylose (~16%) occurred when a 10% slurry of ground straw was heated at 160 °C for 60 min. When hydrothermally (160 °C, 1h) treated straw was not separated into liquid and solid phases but was digested directly with 40 IU/g dry straw of Multifect xylanase, a yield of 16% D-xylose was obtained along with 6% other sugars, namely, 1.4% L-arabinose, 4.2% D-glucose, and 0.8% D-galactose (**Figure 3**). The maximum yield of 16% D-xylose obtained by commercial xylanase hydrolysis was lower than the 19% D-xylose obtained using 0.3 M sulfuric acid under pressure at 123 °C for 28 min.

The desired outcome of enzymic hydrolysis of hydrothermally treated wheat straw, namely, to selectively release D-xylose, was not achieved using four commercial xylanases. L-Arabinose, D-glucose, and lesser amounts of D-galactose accompanied the release of D-xylose, probably because the commercial enzymes contained, besides endo-β-1,4-xylanase and β-1,4-xylosidase, other hydrolase activities (*48, 49*). Still, the xylanase process is preferred over the two acid processes. The enzyme reaction conditions are mild, although the pretreatment of straw requires mechanical and thermal energy. But the lignocellulosic coproduct is not contaminated with mineral acid, and its swollen condition renders it immediately susceptible to cellulase to produce ethanol or ruminant animal feed. The hydrothermal pretreatment (autohydrolysis) of straw in the enzyme process has the potential of producing xylooligosaccharides, which are valued as prebiotics (*45*). Future work is required to (i) optimize the xylanase hydrolysis step with regard to concentrations of reactants and time, (ii) test new xylanases, and (iii) test the coproduct lignocellulose in ruminant animals and ethanol production. Ultimately, the electrochemical method (*11*) to produce xylitol from D-

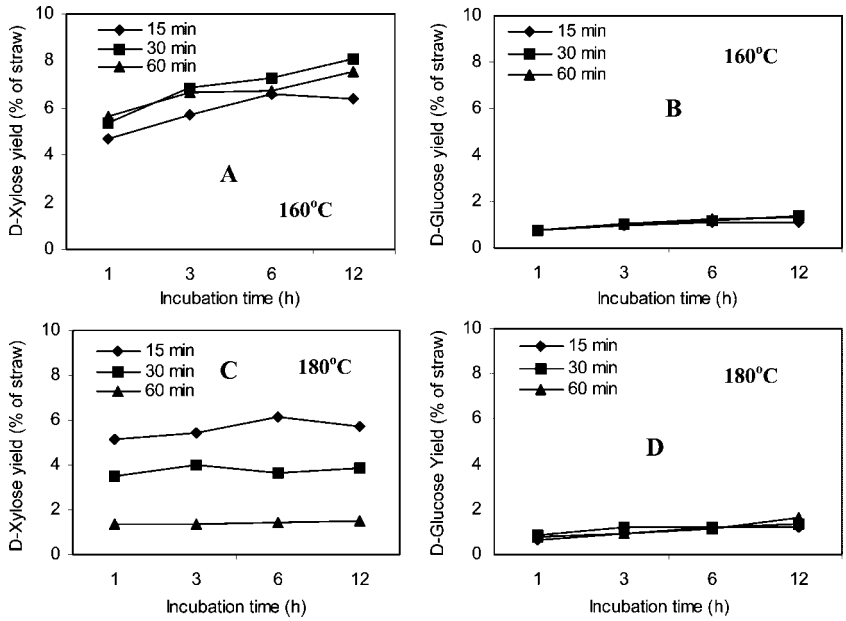

Figure 6. D-Xylose (**A** and **C**) and D-glucose (**B** and **D**) released by *T. reesei* (Multifect^{R}) xylanase hydrolysis of the solid swollen phase from wheat straw hydrothermally treated at 160 and 180 °C.

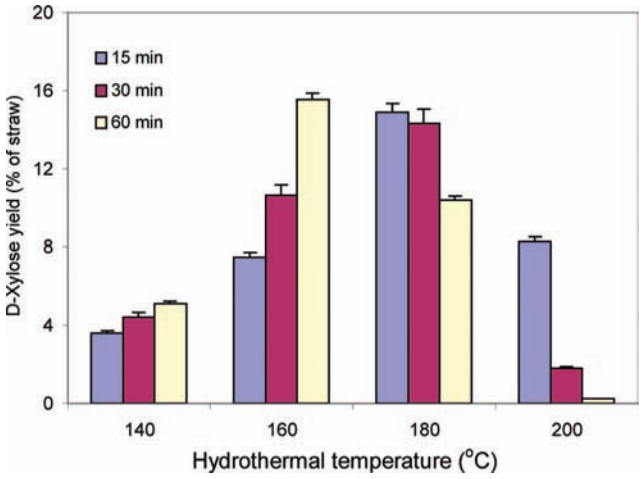

Figure 7. Total yield of D-xylose (combined and free forms) obtained from the liquid phase and summed with the D-xylose released from the solid phase of hydrothermally treated wheat straw. The treated wheat straw mixture was separated into liquid and solid phases, and the phases hydrolyzed with acid and xylanase, respectively, to obtain the total yield of D-xylose from each phase. The monosaccharides in the hydrolyzates were determined by HPAEC-PAD with a coefficient of variance of 3.0%.

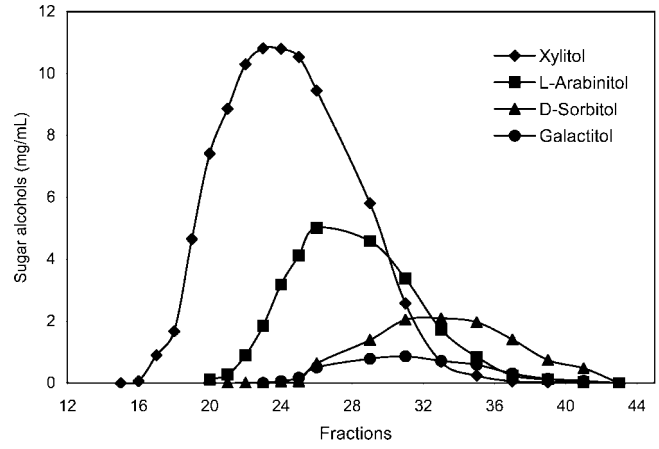

Figure 8. Separation of sugar alcohols at ambient temperature on a strongly basic anion-exchange resin in the hydroxide (OH^-) form with resin bead-size 75–150 μm. The alditols produced from wheat straw were added atop the column at 0.2% based on wet resin and were eluted with water.

glucuronate may well prevail over xylan-hydrolysis methods, unless the biomass containing the xylan yields additional coproducts of substantial value.

Preparation and Isolation of Xylitol from Wheat Straw. A straw hydrolyzate (10% straw in 0.3 M sulfuric acid for 30 min at 123 °C) was chosen to illustrate the preparation and purification of xylitol. The hydrolyzate contained 19.1% D-xylose, 3.1% L-arabinose, 2.2% D-glucose, and 0.7% D-galactose, or ~25% monosaccharides, on the basis of straw. The swollen residue remaining after hydrolysis amounted to 60% of dry straw weight, and it was calculated to contain ~65% cellulose and ~35% lignin on a dry basis. Neutralization of the acid hydrolyzate followed by removal of water under vacuum caused approximately one-half of the sodium sulfate to crystallize. After the removal of the salt, the filtrate was deionized by stirring with mixed-bed ion-exchange resin in the hydrogen ion and hydroxide ion forms, which removed both mineral ions and peptides. HPAEC-PAD assay of the ion-exchanged solution showed ~75% recovery of the monosaccharides upon removal of the resin.

The sugars were reduced to alditols conveniently in the laboratory using sodium borohydride. Removal of inorganic ions from the alditols gave 88% recovery of total alditols. Fractional crystallizations in cold 90% ethanol gave a 10% yield of crystalline xylitol, which contained 95% xylitol and a total of 5% D-sorbitol plus L-arabinitol. Repeated crystallization gave no improvement in the purity of xylitol. A 16% yield of xylitol crystals of unknown purity was reported from a birchwood hydrolyzate (*10*).

Xylitol of 99% purity with 1% L-arabinitol was obtained in ~7% yield from the straw hydrolyzate after chromatography on an open column of a strongly basic anion-exchange resin. The order of elution of the alditols from the column was, from first to last, xylitol, L-arabinitol, D-sorbitol, and galactitol (**Figure 8**). When the alditols were separated as ligands to calcium ions, which were held on a strongly acidic

cationic exchange resin, the order of elution was L-arabinitol, galactitol, xylitol, and D-sorbitol (data not shown), which agrees with the separation reported by Melaja and Hämäläinem (*10*).

The column chromatographic purification of xylitol from other sugar alcohols is facilitated when xylitol elutes first. Resolution of the four sugar alcohols on the basic anion-exchange resin declined when loading of the wet resin increased from ~0.2% to 1.0%. In the future, loading might be increased by improving the resins or reducing the size of the resin beads. Alternatively, a reduced straw hydrolyzate could be fractioned first on a column of strongly acidic resin in the calcium ion form to remove L-arabinitol and galactitol. Then the purified fraction containing xylitol and D-sorbitol could be separated on the strongly basic anion-exchange resin as illustrated in **Figure 8**.

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