XYLO-OLIGOSACCHARIDES PRODUCTION BY AUTOHYDROLYSIS OF CORN FIBER SEPARATED FROM DDGS

Aditya Samala,[a] Radhakrishnan Srinivasan,[a,]* Madhav P. Yadav,[b] Tae-Jo Kim,[c] and Lynn Prewitt [d]

Xylo-oligosaccharides (XOS) are reported to have beneficial health properties, and they are considered to be functional food ingredients. Corn fiber separated from distillers dried grains with solubles (DDGS) could be a valuable feedstock for XOS production. The objective of this study was to determine the efficacy for autohydrolysis to produce XOS using fiber separated from DDGS and to determine the optimum temperature for XOS production. Corn fiber was treated with deionized water in a Parr-reactor, at temperatures ranging from 140 to 220 ^{o}C to produce XOS. The maximum total yield of XOS in the solution was 18.6 wt% of the corn fiber at 180 ^{o}C.

Keywords: DDGS; Oligosaccharides; Corn fiber; Feedstock; XOS

*Contact information: a: Department of Agricultural and Biological Engineering, Mississippi State University, Box 9632, Mississippi State, MS 39762, USA; b: Eastern Regional Research Center, Agricultural Research Service, U. S. Department of Agriculture, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; c: Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, MS 39762, USA; d: Department of Forest Products, Mississippi State University, Mississippi State, MS 39762, USA. * Corresponding author: rs634@msstate.edu*

INTRODUCTION

The need for renewable energy sources has led to a rapid increase in the production of fuel ethanol, as well as its co-product, distillers dried grains with solubles (DDGS). DDGS consists of non-fermentable components of the original grain, such as protein, lipids, and fiber. The effective utilization of DDGS has become important with respect to maintaining the economic viability of the renewable bio-fuel industry. For every 1 kg of corn utilized, nearly 1/3 kg of each of the products ethanol, DDGS, and CO_2 are produced. In 2006, nearly 10 million metric tons of DDGS was produced from this industry. DDGS, which has high protein and fiber contents, is used as livestock feed, mainly as ruminant (cattle) feed. However, with an increase in supply of distillers grains, innovative uses for DDGS are needed to increase its value. Corn fiber separated from distillers dried grains with solubles (DDGS) could be a valuable feedstock for production of xylo-oligosaccharides (XOS), which can be used as a functional food ingredient.

Recently, fiber has been separated from DDGS using a combination of sieving and air classification (the Elusieve process) to produce two beneficial co-products: (1) enhanced DDGS with reduced fiber, increased fat, and protein contents and (2) fiber (Srinivasan *et al.* 2006). Enhanced DDGS from Elusieve processing has lower fiber content and hence, has potential to be used at higher inclusion levels in non-ruminant animal diets. Currently, fiber separated from DDGS is believed to have limited use as

feed for ruminant animals (dairy and beef cattle). XOS production could increase the value of the fiber.

Polymerized monosaccharides having a degree of polymerization (DP) between 2 and 10 are defined as oligosaccharides (Nakakuki 1993). Oligosaccharides that cannot be digested in the human stomach and small intestine are considered as functional food ingredients with potential to reduce the risk and possibility of heart diseases, bacterial/ viral infections, cancer, diabetes, hepatitis, emphysema, and cranial and muscular neurological diseases (Hakomori and Kannagi 1983; Faissner *et al.* 1994; Gibson 2004; Rivas *et al.* 2002; Chu and Whittaker 2004; Kawakubo *et al.* 2004; Ohtsubo and Marth 2006). Due to their various health benefits, oligosaccharides are used in pharmaceutical and food industries. Commonly used oligosaccharides are fructo-oligosaccharides, malto-oligosaccharides, galacto-oligosaccharides, and XOS.

XOS are xylose-based oligomers linked by β-1,4 bonds; they contain variable amounts of substituted groups such as acetyl, phenolic, and uronic acid. XOS are considered to be prebiotics. Prebiotics are defined as non-digestible food ingredients that benefit the host by stimulating the growth and activity of a limited number of bacteria, such as *Bifidobacterium* species, in the colon (Gibson and Roberfroid 1995). Prebiotics have applications in pet foods, human foods, and animal feeds.

Different methods used for the production of xylo-oligosaccharides are: enzymatic hydrolysis, alkali/acid hydrolysis, and autoydrolysis of carbohydrate polymer. In enzymatic hydrolysis, enzymes such as endoxylanases, β-xylosidases, and arabino-furanosidases are used to break the xylan linkages to produce XOS. Enzymatic hydrolysis typically takes a longer time for completion than other methods. In acid/alkali hydrolysis methods, a dilute solution of acid or base is used to treat the substrates, typically at ambient temperature, to produce XOS. In the autohydrolysis method, water is added to the substrate and the mixture is heated to the range 100 to 250 ºC in an enclosed vessel to produce XOS. No prior work has been conducted on autohydrolysis of fiber separated from DDGS. Even though XOS production by autohydrolysis has been evaluated for some other agricultural materials such as corn cobs and wood, those results are not sufficient to determine the optimum conditions required for autohydrolysis of fiber separated from DDGS, because this is a different material in terms of composition and origin (Carvalheiro *et al.* 2004; Nabralatz *et al.* 2007). The objective of this study was to determine the efficacy for autohydrolysis to produce XOS using fiber separated from DDGS and determine the optimum temperature for production of XOS.

EXPERIMENTAL

Fiber Separation from DDGS

DDGS was procured from a local feed mill and processed to separate fiber using the Elusieve pilot-plant at Mississippi State University (Srinivasan *et al.* 2009). Quantity of fiber separated was 4% of the weight of DDGS. The fiber used in this study was the large size fiber fraction (size > 868 µm). Quantity of large size fiber fraction was 2% of the weight of DDGS. The fiber material was stored in vacuum-sealed bags in a refrigerator at 5 ^{o}C until used.

Determination of Corn Fiber Composition

Three replicates of the corn fiber material were sent to Integrated Paper Services, Inc., Appleton, WI for determination of corn fiber composition. The samples were milled to approximately 40-mesh. Prior to carbohydrate and lignin analysis, samples were extracted with dichloromethane (DCM) in a soxhlet apparatus to remove substances such as waxes, fats, resins, phytosterols, and non-volatile hydrocarbons. The percent of extractives of each sample was calculated based on its oven-dried weight. Extractives content for corn fiber material was 6.7%. The carbohydrate and lignin content determination of three dichloromethane extracted samples was done in duplicate. Lignin content was determined according to method described in Effland (1977). Approximately 300 mg of sample was hydrolyzed with acid and filtered. The acid-insoluble residue was oven-dried and weighed to calculate the percent of lignin content. The acid-soluble portion of each sample was neutralized, reduced, acetylated, and their carbohydrate composition determined according to TAPPI Test Method T249 Cm-00 using a Flame Ionization Detector-Gas Chromatograph (FID-GC).

Determination of Carbohydrate Composition of Residue from Autohydrolysis

Three replicates of residue materials were analyzed. Samples were extracted with hexane in a soxhlet apparatus. The extractives comprised 15.4% of the residue. Carbohydrate content (glucan, xylan, galactan, and arabinan) of the remaining material was determined using procedure similar to that used for corn fiber (previous section) at the USDA lab in Wyndmoor, PA.

Auto-Hydrolysis of Fiber

The autohydrolysis of fiber was conducted in a 750 mL Parr reactor (model 4843, Parr Instruments Co., Moline, Illinois, USA) (Fig. 1). The reactor (fitted with a six bolt metal cover) was heated with temperature control. In each batch, the Parr reactor was filled and loaded with 10 grams of corn fiber and 90 mL of deionized water. The holding time after the desired temperature was reached was 15 min. The treatment of fiber separated from DDGS samples was carried out at a desired set of temperatures between 140 and 220 ^{o}C. The time required to reach 140 to 220 °C was 14 to 45 min. The reaction mixture was not stirred during the heating period. There was some charring of corn fiber on the surface of the reactor cylinder at higher temperatures (200 and 220°C). Autohydrolysis was carried out in three replicates at each temperature, except for 200 ^{o}C and 220 ^{o}C.

The reaction mixture was filtered by gravity filtration using filter paper (Fisherbrand, USA, catalogue no. 09-801E, particle retention 5 to 10 µm) and a size P5 funnel. The filtrate was further filtered by a vacuum filtration system using a glass fiber prefilter (Millipore, USA) on a Buchner funnel. The reaction mixture was filtered twice to obtain particle-free solution for HPLC analysis. The solid product was thoroughly washed with deionized water ranging from 100 mL to 120 mL to ensure complete oligosaccharides removal from residue and dried at room temperature. The washing was collected in a bottle, labeled as original liquor, and stored in the refrigerator at 0 ^{o}C.

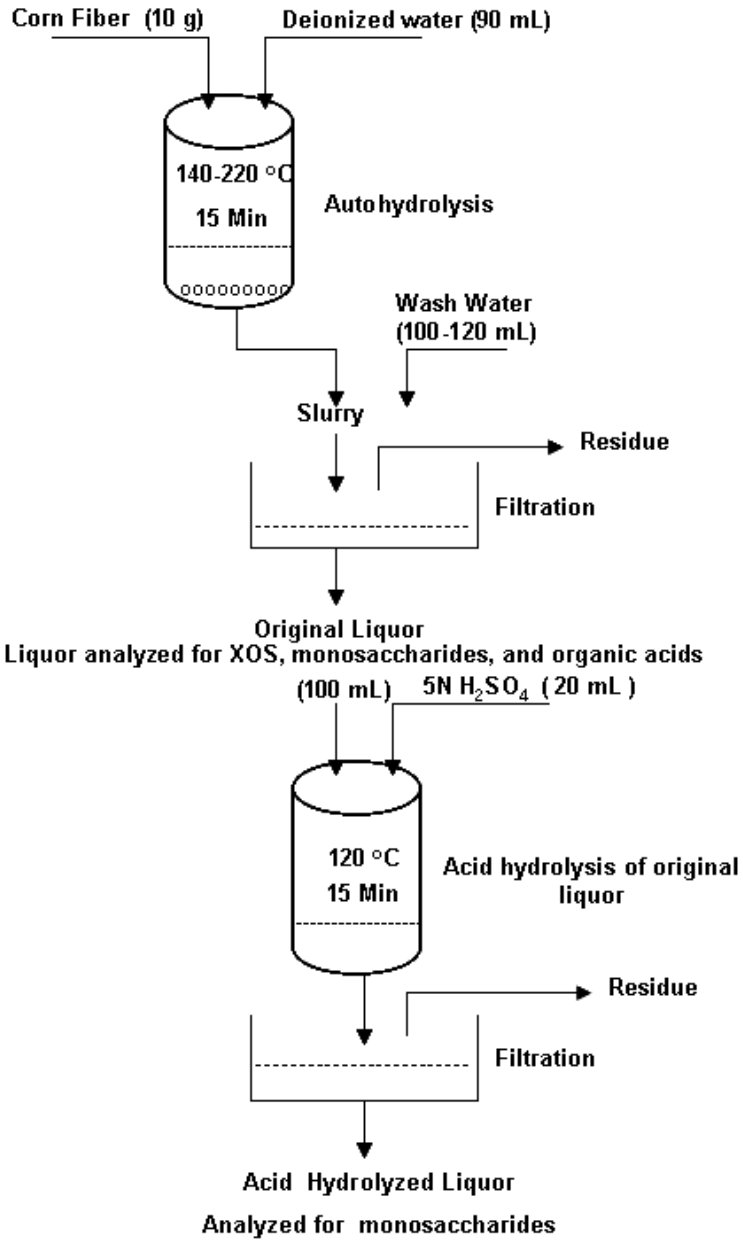

Fig. 1. Schematic for autohydrolysis of corn fiber to produce XOS and acid hydrolysis of liquor

Acid Hydrolysis of Liquor from Autohydrolysis

The original liquor (100 mL) obtained after autohydrolysis reaction of fiber was mixed with 20 mL of 5N H_2SO_4 and heated at 120 ^oC for 45 minutes to hydrolyze XOS to their monomeric sugars (Fig. 1) using NREL (National Renewable Energy Laboratory) procedure NREL/TP-510-42623. The acid hydrolyzed solution was filtered by vacuum filtration on a Buchner funnel to remove insoluble materials. Acid-hydrolyzed liquor was quantified for monosugars content to provide an indirect means to verify results of

autohydrolysis. Monosugars content was determined using an HPLC method (see next) at Mississippi State University.

Quantification of XOS, Monosaccharides, and Acids using HPLC

The procedure used for quantification of XOS was similar to that used by earlier researchers (Carvalheiro *et al.* 2004; Nabralatz *et al.* 2007). An aliquot from the acid-hydrolyzed sample solution was further filtered using 0.22 μm syringe filters into a 2 mL vial (Agilent, USA) for sugar analysis. Sugars were analyzed by high-performance liquid chromatography (HPLC) using an Agilent 1200 series HPLC System device (Agilent, USA) equipped with a refractive index detector. The monosaccharide content of both the original liquor as well as acid hydrolyzed liquor was determined by the HPLC unit equipped with a Bio-Rad HPX 87 P (300 X 7.8 mm) column at 80 ^{o}C and a guard column (Bio-Rad Laboratories, USA) by injecting 20 μL of the sample solution and eluting the column with HPLC grade water (Sigma Aldrich, USA). The standard sugars used for identification and quantification were glucose, xylose, arabinose, galactose, and mannose (Sigma Aldrich, USA).

The XOS in the original liquor were analyzed by the HPLC device equipped with a Bio-Rad HPX 42 A column at 80 ^{o}C and a guard column (Bio-Rad Laboratories, USA) by eluting the column with HPLC grade water (Sigma Aldrich, USA) at a flow-rate of 0.6 mL/min. The XOS standards used were xylobiose, xylotriose, xylotetrose, xylopentose, and xylohexose along with a monomeric xylose (Megazymes, Ireland). The acidic components and sugar degradation products present in the original liquid were analyzed by HPLC equipped with a Bio-Rad HPX 87 H (300 X 7.8 mm) column at 80 ^{o}C and a guard column (Bio-Rad Laboratories, USA) by eluting with 0.005M H_2SO_4 at a flow rate of 0.6 mL/min. Standard acids used were acetic acid, formic acid, and levulinic acid. Degradation compounds used were hydroxymethyl furfural (HMF) and furfural (Sigma Aldrich, USA).

RESULTS AND DISCUSSION

Moisture content of the corn fiber was 12.4% on a wet basis. The carbohydrate composition of the corn fiber was glucan 18.0%, xylan 16.8%, arabinan 8.8%, mannan 0.8%, galactan 3.0%, and lignin content 1.3%, on wet basis. Protein content of corn fiber separated from DDGS was 12.4% on wet basis. Thus, the glucan content represented 18.0% and hemicelluloses content, comprising xylan, arabinan, galactan, and mannan chains, represented 29.4.% in the corn fiber. Based on the composition of corn fiber, the maximum expected amounts of glucose, xylose, arabinose, galactose, and mannose monosugars were 2.0 g, 1.9 g, 1.0 g, 0.3 g, and 0.0 g, respectively. Thus the total expected maximum amount of monosugars based on corn fiber composition was 5.2 g.

The liquor obtained after autohydrolysis of fiber in the Parr-reactor at temperatures ranging from 140 to 220 ^{o}C consisted mostly of a mixture of xylose oligomers with some free arabinose and glucose (Tables 1 and 2). Representative chromatograms for quantification of monosugars in the original liquor, XOS in the original liquor, and XOS standards are shown in Fig. 2, Fig. 3, and Fig. 4, respectively.

Similar to observations of Aoyama (1996), Garrote *et al.* (1999), and Carvalheiro *et al.* (2004), production of XOS depended upon temperature. As the temperature was increased from 180 to 220 ^{o}C, the XOS production decreased, but the production of acetic acid and degradation compounds (HMF, furfural) increased. The maximum amount of XOS from the original liquor was obtained at temperature 170 to 180 ^{o}C (Table 1). The amount of XOS in the original liquor increased with increasing temperature up to 180 ^{o}C, but it decreased on further increase of temperature. The estimated xylose content in the acid hydrolyzed liquor based on hydrolysis of measured XOS, was higher than measured xylose content until a temperature of 150 ^{o}C. This may be due to co-elution of other sugar-oligosaccharides with XOS in the Aminex 42-A column, which was also observed when analyzing standard samples; cellobiose co-eluted with xylotriose. As the temperature was increased, there was an increase in the breakdown of other sugar-polymers (glucan, arabinan, and galactan) into monomers, giving rise to higher levels of monomers (arabinose, galactose, and glucose) at higher temperatures up to 180 ^{o}C (Table 2). This degradation of carbohydrate polymers into monomers would result in a decrease of other-sugar oligosaccharides, which probably led to lesser co-elution of XOS at higher temperature. Lesser co-elution at higher temperatures can be inferred from xylose content in acid hydrolyzed liquor being higher than the minimum xylose content expected from hydrolysis of measured XOS in the original liquor at higher temperatures (Table 2). Thus, the measured XOS values at temperatures higher than 160 ^{o}C are expected to be closer to actual values. In further studies that are underway, we are pursuing preparative HPLC method to purify and quantify the exact amounts of individual oligomer compounds of xylan, arabinan, galactan, and cellulose.

In order to verify results, a component-wise mass balance was carried out for the autohydrolysis process at a single temperature (180 ^{o}C) (Table 3).

Table 1. XOS, Acetic Acid and Degradation Products Contents (mg) of Original Liquor

Temp (ºC)	XOS					Acetic Acid	Degradation compounds	
	Xylotriose	Xylotetrose	Xylopentose	Xylohexose	Total XOS		HMF	Furfural
140	1154	87	72	0	1314	47	0	0
150	1365	105	55	0	1524	50	0	0
160	1215	104	97	0	1415	72	0	0
170	1483	142	146	20	1790	534	0	0
180	1645	0	221	0	1865	640	0	0
200*	535	0	0	0	535	1355	524	1632
220*	190	0	0	0	190	1723	839	1705

Results are means of three replicates; HMF- Hydroxymethylfurfural
* Results are means of only one replicate
The range of coefficients of variation for xylotriose, xylotetrose, xylopentose, xylohexose and acetic acid were 6.9 - 13.3%, 86.6 - 99.3%, 15.6 – 86.6%, 0.0 – 173.2%, 6.1 – 73.3%, respectively.

Table 2. Monosaccharide Content (mg) of Acid Hydrolyzed Liquor and Original Liquor

Temp (ºC)	Monosaccharides measured in acid hydrolyzed liquor				Monosaccharides measured in original liquor				Minimum Xyl Expected in Acid Hydrolyzed Liquor Based on XOS values from oligosaccharide column
	Xyl	Glu	Gal	Ara	Xyl	Glu	Gal	Ara	
140	438	1446	299	433	366	656	0	435	1746
150	930	1477	238	647	436	661	115	1002	2104
160	2729	1525	456	1018	636	1023	213	1398	2442
170	3778	1269	0	1152	1011	1275	447	1647	3199
180	5096	1291	0	1066	1312	1424	700	1578	4096
200*	1385	1262	0	0	1072	1294	639	875	1156
220*	479	403	0	99	0	405	0	0	206

Results are means of three replicates; Xyl – Xylose, Glu – Glucose, Gal – Galactose, Ara – Arabinose; The range of coefficients of variation for glucose, glucose, xylose, arabinose, galactose were 1.4 – 9.9%, 3.6 - 9.3%, 5.2 - 60.9%, 13.0 – 69.7%, respectively.
* Results are means of only one replicate.
§ Calculated using values from table 1 as (xylose + xylobiose * 300/282 + xylotriose * 450/414 + xylotetrose * 600/546 + xylopentose * 750/678 + xylohexose * 900/810)

Table 3. Component-Wise Mass Balance for Autohydrolysis Process at 180 ºC

Material	Glucose (g)	Xylose (g)	Galactose (g)	Arabinose (g)	Total (g)
Corn fiber (Input)	2.00	1.91	0.34	1.00	5.25
Original liquor (sugars)	1.42	1.31	0.70	1.58	5.01
Original liquor (sugars from oligosaccharides)	0.00	1.86	0.00	0.00	1.86
Residue	0.00	0.00	0.00	0.00	0.00
Total output	1.42	3.17	0.70	1.58	6.87
% Difference between input and output	-29%	66%	106%	58%	31%

Compositional analysis showed that residue from autohydrosis did not contain any carbohydrates (glucan, xylan, galactan, and arabinan). It was found that the overall material balance error was 31% and the component wise error was -29% to 66% for all sugars except galactose. The mass balance error was higher (106%) for galactose perhaps because of the low quantities of galactose in the materials.

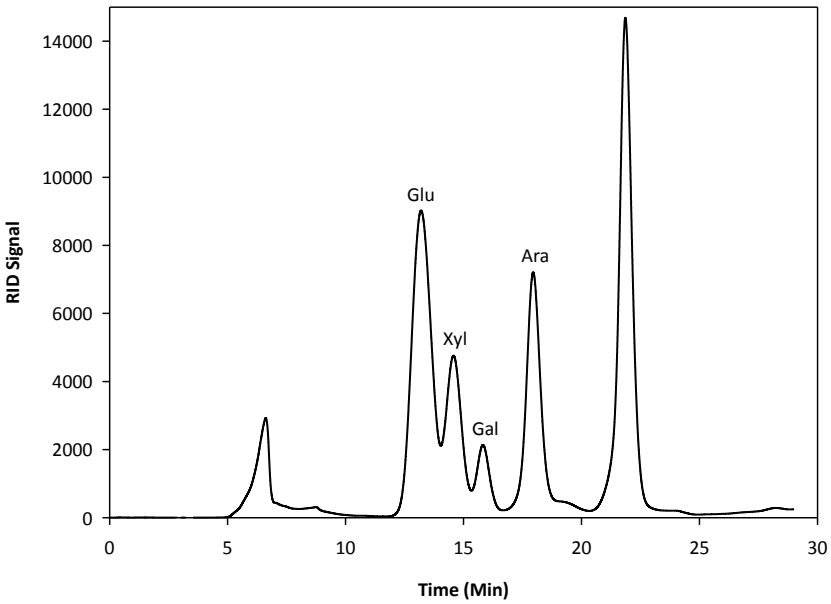

Fig. 2. HPLC analysis of original liquor obtained by autohydrolysis of corn fiber at 170 °C, for quantification of monosaccharide using Bio-Rad HPX 87 P (300 X 7.8 mm) column at 80 °C by eluting with HPLC grade water. Actual run time was 60 min, but the chromatogram is truncated in the figure to improve clarity. Glu – glucose, Xyl – xylose, Gal – Galactose Ara – arabinose. Retention times for Glu, Xyl, Gal and Ara were 13.15, 14.52, 15.57 and 17.45 min, respectively

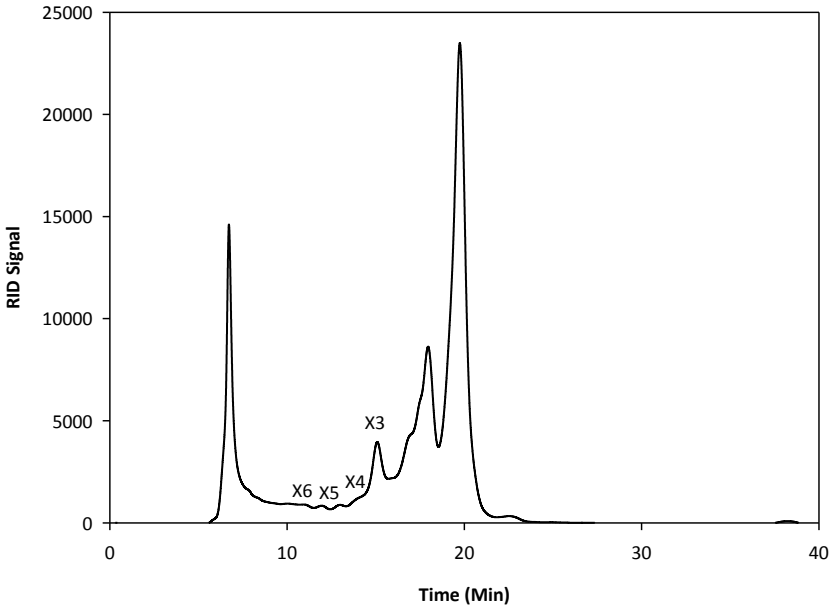

Fig. 3. HPLC analysis of original liquor obtained by autohydrolysis of corn fiber at 170 °C for quantification of xylooligosaccharides (XOS) using Bio-Rad HPX 42 A column at 80 °C by eluting with HPLC grade water. Actual run time was 60 min, but the chromatogram is truncated in the figure to improve clarity. X3: Xylotriose, X4: Xylotetrose, X5: Xylopentose, X6: Xylohexose. Retention times for X3, X4, X5 and X6 were 14.98, 12.88, 11.56 and 11.01 min, respectively

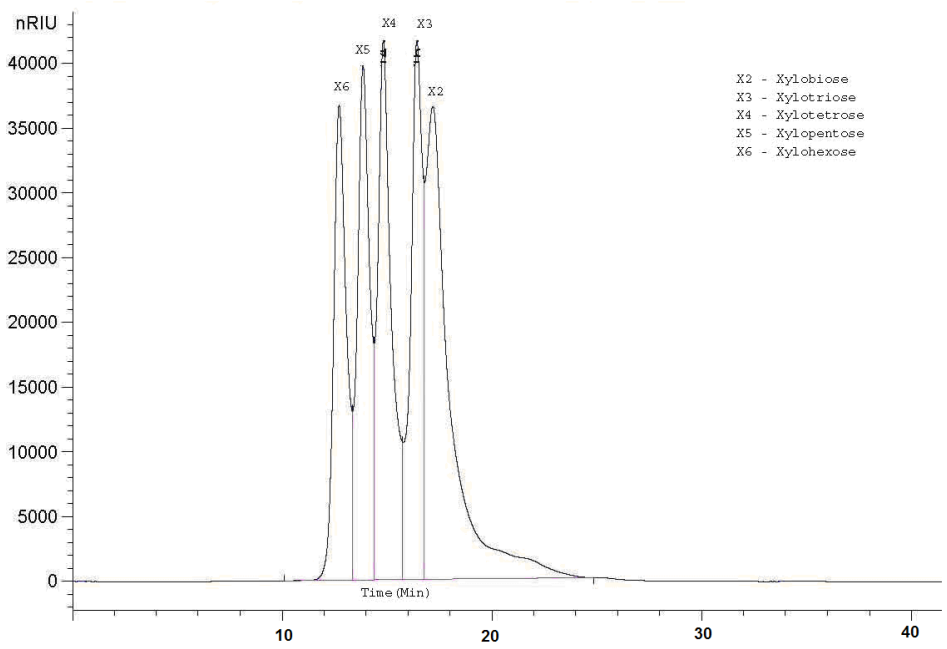

Fig. 4. HPLC analysis of XOS standards for quantification of xylooligosaccharides (XOS) using Bio-Rad HPX 42 A column at 80 ^{o}C by eluting with HPLC grade water. Actual run time was 60 min, but the chromatogram is truncated in the figure to improve clarity. X2: Xybiose, X3: Xylotriose, X4: Xylotetrose, X5: Xylopentose, X6: Xylohexose

As temperature was increased, the breakdown of xylan polymer increased. There was an increase in the xylose content from 316 to 2064 mg in the original liquor as temperature was increased from 140 to 180 ^{o}C (Table 1). The maximum yield of XOS was 18.6% of corn fiber at 180 ^{o}C, containing mainly xylotriose (1645 mg) and xylopentose (221 mg). The XOS produced at 170 ^{o}C contained mainly a mixture of xylotriose (1483 mg), xylotetrose (142 mg), xylopentose (146 mg), and xylohexose (20 mg). At 170 ^{o}C the original liquor showed the presence of a mixture of xylotriose, xylotetrose, and xylopentose. But at 180 ^{o}C the original liquor showed a mixture of only xylotriose and xylopentose, which may be due to co-elution of xylotetrose and xylohexose with xylotriose/xylopentose. Xylobiose was not detected in any of the original liquors, which also is perhaps due to co-elution. It is evident from data presented in Table 1 that hemicellulose certainly was hydrolyzed on heating to form xylo-oligosaccharides. Results of the steam explosion work performed by Nunes and Pourquie (1996) were in agreement with this study in terms of decreasing oligomer/monomer concentrations at high temperatures and pressures due to thermal degradation.

The breakdown of arabinan into monomer seems to be complete at 170 ^{o}C, as indicated by the highest arabinose content (1647 mg) in the original liquor at 170 ^{o}C. At higher temperatures, the arabinose content in original liquor decreased because of its conversion into other compounds such as furfural (Kootstra *et al.* 2009; Table 2). The breakdown of galactan and cellulose to their respective monomers seemed to be maximized at 180 ^{o}C, as indicated by the highest galactose and glucose contents (700 and 1424 mg, respectively) in the original liquor at 180 ^{o}C. Contents of arabinose, galactose,

and glucose monomers in acid hydrolyzed liquor were lower at some conditions, especially at higher temperatures, than respective monomer contents in original liquor. The loss of these sugars during acid hydrolysis at high temperature is more likely due to their conversion into known compounds such as furfural, hydroxymethyl furfural, and some other un-characterized degradation products (Table 2). For example, degradation of arabinose at higher temperatures has been reported by Kootstra *et al.* (2009). The HPLC analysis results of XOS and monosaccharides at 170/180 ^{o}C (temperatures at which non-xylan sugars break down completely into monomers) were comparable to that expected based on composition of the original fiber. The validity of HPLC analysis results was verified by comparing the total monosugars content in the original liquor with the maximum expected monosugars content based on the carbohydrate content of the corn fiber. Total monosugars content in original liquor at 140, 150, 160, 170, and 180 ^{o}C were 1.5, 2.4, 3.3, 4.4, and 5.0 g, respectively, which were less than the maximum expected monosugars content of 5.2 g based on corn fiber composition. Thus, the HPLC results were in agreement with the original corn fiber composition.

The maximum XOS yield obtained in this study (18.6% of feedstock) was comparable to maximum XOS yield observed by earlier autohydrolysis studies. The maximum XOS yield from brewery's spent grain (BSG) was 14.1% (Carvalheiro *et al.* 2004). The maximum XOS yield from almond shells was 15.7% (Nabarlatz *et al.* 2007).

The original liquor had no traces of formic acid and levulinic acid, which are formed on the degradation of HMF and furfural compounds (Dunlop 1948; Ulbricht *et al.* 1984). As temperature increased, the acetic acid content in original liquor also increased, perhaps due to de-acetylation of arabinoxylan hemicellulose (Table 1).

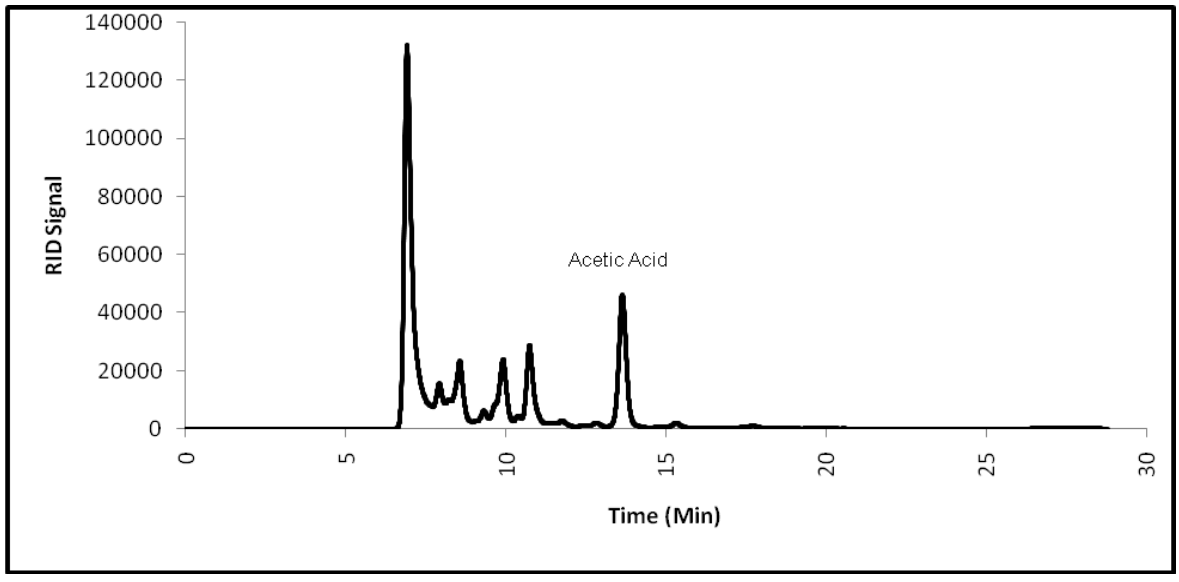

Fig. 5. HPLC analysis of original liquor obtained by autohydrolysis of corn fiber at 170 °C for quantifications of sugar degradation and acidic components using Bio-Rad HPX 87 H (300 X 7.8 mm) column at 80 °C by eluting with 0.005M H_2SO_4 . Furfural and HMF were not present at 170 °C. Furfural and HMF were present at temperatures above 200 °C. Retention times for HMF and furfural were 30.44 and 46.70 min, respectively. Actual run time was 60 min, but the chromatogram is truncated in the figure to improve clarity

A representative chromatogram showing quantification of acetic acid and degradation products is given in Fig. 5. Acetic acid production increased from 47 mg to 1723 mg as the temperature increased from 140 to 220 ^{o}C, and the formation of HMF and furfural started at 200 ^{o}C. It is beneficial that HMF and furfural were not present at 180 ^{o}C, when the XOS production was at its maximum. In industrial scale production of XOS, original liquor from autohydrolysis would need to be purified by separating the monosugars using membranes and decolorized by adsorption methods.

CONCLUSIONS

1. Xylo-oligosaccharides (XOS) can be produced by autohydrolysis of fiber separated from distillers dried grains with solubles (DDGS).
2. The production of XOS increased as the temperature increased from 140 to 180 ^{o}C, and XOS production was lower at temperatures of 200 to 220 ^{o}C.
3. The maximum total XOS yield was 18.6 % of corn fiber, which was obtained at 180 ^{o}C. The original liquor had no traces of formic acid and levulinic acid.
4. As temperature increased, the acetic acid content in original liquor increased. Formation of HMF and furfural started at 200 ^{o}C.

ACKNOWLEDGMENTS

We thank Kaleb Smith, Bubba Trammell, and Ravi Challa for their technical assistance and Sustainable Energy Research Centre (SERC) for providing partial funding for this project. We thank Dr. Kevin Hicks, Research Leader, Sustainable Biofuels and Co-Products, ERRC, ARS, USDA, for critically reviewing the manuscript.

REFERENCES CITED

Aoyama, M., (1996). "Steaming treatment of bamboo grass. II. Characterization of solubilized hemicellulose and enzymatic digestibility of water-extracted residue," *Cell. Chem. Technol.* 30(5), 385-393.

Carvalheiro, F., Esteves, M. P., Parajó, J. C., Pereira, H., and Gírio, F. M. (2004). "Production of oligosaccharides by autohydrolysis of brewery's spent grain," *Bioresource Technol.* 91(1), 93-100.

Chu, V. C., and Whittaker, G. R. (2004). "Influenza virus entry and infection require host cell N-linked glycoprotein," *PNAS USA* 101(52), 18153-18158.

Dunlop, A. P. (1948). "Furfural formation and behaviour," *Ind. Eng. Chem.* 40(2), 204-209.

Effland, M. J. (1977). "Modified procedure to determine acid-insoluble lignin in wood and pulp," *Tappi* 60 (10), 143-144.

Faissner, A., Clement, A., Lochter, A., Streit, A., Mandl, C., and Schachner, M. (1994). "Isolation of a neural chondroitin sulfate proteoglycan with neurite outgrowth promoting properties," *J. Cell Biol.* 126(3), 783-799.

Garrote, G., Dominguez, H., and Parajo, J. C. (1999). "Mild autohydrolysis: An environmental friendly technology for xylo-oligosaccharides production from wood," *J. Chem. Technol. Biotechnol.* 74(11), 1101-1109.

Gibson, G., (2004). "Fibre and effects on probiotics (the prebiotic concept)," *Clin.Nutr. Supplements* 1(2), 25-31.

Gibson, G. R., and Roberfroid, M. B. (1995). "Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics," *J. Nutr.* 125(6), 1401-1412.

Hakomori, S., and Kannagi, R. (1983). "Glycosphingolipids as tumor associated and differentiation makers," *J. National Cancer Institute* 71(2), 231-251.

Kawakubo, M., Ito, Y., Okimura,Y., Kobayashi, M., Sakura, K., Kasama, S., Fukuda, M. N., Fukuda, M., Katsuyama, T., and Nakayama, J. (2004). "Natural antibiotic function of a human gastric mucin against Helicobacter pylori infection," *Science* (305), 1003-1006.

Kootstra, A. M. J., Mosier, N. S., Scott, E. L., Beeftink, H. H., and Sanders, J. P. M. (2009). "Differential effects of mineral and organic acids on the kinetics of arabinose degradation under lignocellulose pretreatment conditions," *Biochem. Eng. J.* 43(2009), 92-97.

Nakakuki, T. (1993). "Oligosaccharides: Production, properties and applications," *Japanese Technology Reviews* Vol. 3(2), Gordon and Breach, Switzerland.

Nabarlatz, D. A., Ebringerova, D., and Montane. (2007). "Autohydrolysis of agricultural by-products for the production of xylo-oligosaccharides," *Carb. Polym.* 69(1), 20-28.

Nunes, A. P., and Pourquie, J. (1996). "Steam explosion pretreatment and enzymatic hydrolysis of eucalyptus wood," *Bioresource. Technol.* 57(2), 107-110.

Ohtsubo, K., and Marth, J. D. (2006). "Glycosylation in cellular mechanisms of health and disease," *Cell* 126(5), 855-867.

Rivas, B., Domínguez, J., Domínguez, H., and Parajó, J. (2002). "Bioconversion of posthydrolysed autohydrolysis liquors: An alternative for xylitol production from corn cobs," *Enzyme Microb. Tech.* 31(4), 431-438.

Srinivasan, R., Singh, V., Belyea, R. L., Rausch, K. D., Moreau, R. A., and Tumbleson, M. E. (2006). "Economics of fiber separation from Distillers Dried Grain with Solubles (DDGS) using sieving and elutriation," *Cereal Chem.* 83(4), 324-330.

Srinivasan, R., To, F., and Columbus, E. (2009). "Pilot scale fiber separation from distillers dried grains with solubles (DDGS) using sieving and air classification," *Bioresource Technol.* 100(14), 3548-3555.

TAPPI T 249 cm-00, "Carbohydrate composition of extractive-free wood and wood pulp by gas-liquid chromatography," in: 2002-2003 TAPPI Test Methods, 2002, TAPPI Press, Atlanta, GA, USA.

Ulbricht, R. J., Northup, S. J., and Thomas, J. A. (1984). "A review of 5-hydroxymethylfurfural (HMF) in parental solutions," *Fundam. Appl. Toxicol.* 4(5), 843-853.

Article submitted: September 8, 2011; Peer review completed: Oct. 6, 2011; Revised version received: April 30, 2012; Accepted: May 2, 2012; Published: May 25, 2012.