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Comparison of xylo-oligosaccharides production by autohydrolysis of fibers separated from ground corn flour and DDGS

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A B S T R A C T

Xylo-oligosaccharides (XOS) are known to have beneficial health properties, and are considered to be functional food ingredients. The objective of this study is to compare corn fibers separated from ground corn flour and distillers dried grains with solubles (DDGS) for XOS yield and optimum autohydrolysis conditions. Based on the initial xylan content, the fiber separated from ground corn flour (FC) resulted in higher XOS yield (71.5%) than the fiber separated from DDGS (FD) (54.6%) at the maximum XOS production conditions. XOS produced were mainly xylobiose and xylotriose. Based on total initial material also, FC resulted in higher XOS yield (8.9%) than FD (8.0%), based on total original masses. Thus, fiber separated from ground corn flour would be a better feedstock for production of XOS than fiber separated from DDGS. The conditions for maximum XOS production from FD and FC were 180 °C with 20 min hold-time and 190 °C with 10 min hold-time, respectively.

Keywords: Autohydrolysis; Corn fiber; DDGS; Ground corn flour; Xylo-oligosaccharides

1. Introduction

Corn is the most widely produced grain in the United States of which about 52% was used as animal feed, 37% for ethanol production (fuel and beverage) and the remaining 11% for the production of food products (ERS, 2012). In the dry grind process, corn processing results in fuel ethanol, distillers dried grains with solubles (DDGS) and carbon dioxide. Ground corn and DDGS are major ingredients in swine and poultry diets. Ground corn inclusion levels in poultry and swine diets are typically 65% and 50%, respectively. DDGS inclusion level in poultry and swine diets is typically 8% and 30%, respectively. Removal of fiber from feed ingredients increases nutritional value for non-ruminants (swine and a poultry), which do not digest fiber well. Fiber was separated from DDGS and ground corn flour to increase the nutritional value for non-ruminants, using the Elusieve process, which is a combination of sieving and elutriation (air classification) (Srinivasan et al., 2009). It is envisaged that Elusieve process will be used in feed mills to separate fiber from ground corn flour as well as DDGS. Elusieve process removes approximately 10% by weight of corn and 15% by weight of DDGS as fiber. Thus, there will be a significant quantity of fiber separated from corn, in addition to the fiber separated from DDGS.

Fiber is rich in hemicellulose and cellulose, which can be further broken down into oligosaccharides by hydrolysis methods. The present work deals with production of xylo-oligosaccharides (XOS) from corn fiber for the purpose of using

Abbreviations: DDGS, distillers dried grains with solubles; FC, fiber separated from ground corn flour; FD, fiber separated from DDGS; HMF, hydroxymethyl furfural; XOS, xylo-oligosaccharides.

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XOS as a prebiotic. Oligosaccharides are sugar molecules with degree of polymerization (DP) between 2 and 10 and can be classified as digestible or non-digestible based on the physiological properties (Nakakuki, 1993). Mussatto and Mancilha (2007) reported that non-digestible oligosaccharides are fermentable substances in large intestine that benefit human health and are termed as 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.

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 sugar oligomers produced by the hydrolysis of xylan, the major component of plant hemicelluloses. XOS has been produced from corncobs, almond shells, olive stones, wheat straw, barley straw (Nabarlatz et al., 2004) and other materials by hydrolysis methods and XOS are considered to be prebiotics (Gibson and Roberfroid, 1995).

Fiber separated from DDGS is a good feedstock for producing XOS (Samala et al., 2012). XOS production would be influenced by feedstock. There is a need to compare XOS yield of fiber separated from ground corn flour with fiber separated from DDGS and identify the optimum process parameters for autohydrolysis of fiber separated from ground corn flour. This would help XOS producers identify their preferred feedstock and the optimum processing parameters.

In autohydrolysis method, water is added to the substrate and the mixture is heated in temperature range 150–220 °C in an enclosed vessel to produce XOS. This process can be subjected to different reaction times (hold time) of 5, 10, 20 and 30 min. The objective of this study was to determine the yield of XOS from corn fiber separated from ground corn flour and DDGS at each temperature and hold time, determine the optimum conditions (temperature and hold time) for autohydrolysis and characterize the produced liquor for oligomers, monosacharidess, acids and degradation products. The XOS yield from autohydrolysis of fiber separated from ground corn flour was compared with that for fiber separated from DDGS.

2. Materials and methods

2.1. Fiber separation from DDGS and ground corn flour

Similar to the work performed by Samala et al. (2012), 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. Fiber separated from DDGS was referred to as "FD".

Yellow-dent corn grain procured from the farmers' cooperative (Starkville, MS) was hammer milled using a 3.2 mm retainer screen (8/64 inch screen) in the hammer mill as described by Pandya and Srinivasan (2012). The Elusieve process was similar to that used by Pandya and Srinivasan (2012). Large sized fiber (>1532 μm) was used for this study. Fiber separated from ground corn flour was referred to as "FC". The fiber materials were stored in vacuum-sealed bags in a refrigerator at 5 °C until used.

2.2. Determination of lignocellulosic content of fiber separated from DDGS

Procedure used for the determination of lignocellulosic content of fiber was similar to that described by Samala et al. (2012). Three replicates of the FD material were sent to Integrated Paper Services, Inc., Appleton, WI for determination of corn fiber composition. Extractives content for FD was 6.7%, based on total mass of FD. The carbohydrate and lignin content determination of three dichloromethane extracted samples was done in duplicate.

2.3. Determination of cellulosic contents of residue from autohydrolysis and fiber separated from ground corn flour

The cellulose and hemicellulose contents of samples other than FD were determined at Mississippi State University. Lignin content was not determined for these samples. The samples were milled to approximately 40-mesh. Prior to carbohydrate analysis, samples were extracted with methylene chloride in a soxhlet apparatus to remove extractives (soluble in CH_2Cl_2). Extractives content of FC was 1.8%, based on total mass of FC. Extractives content of residues from FD autohydrolysis was in the range of 12.6–35.2%, based on total mass of residue. Extractives content of residues from FC autohydrolysis was in the range of 6.6–17.8%, based on total mass of residue.

The compositional determination of extracted samples was similar to the results reported by Wang et al. (2011) based on the NREL method for "Determination of Structural Carbohydrates and Lignin in Biomass" except that lignin was not measured in this work. "The sample (0.3 g) was treated with 72% H_2SO_4 for 1 h. The resulting mixture was hydrolyzed by adding 84 mL of water and autoclaving at 121 °C for 1 h. The resultant hydrolysis solution was then filtered through the crucibles with a filtering disk to separate the filtrate and residue. The filtrate was used to estimate the content of cellulose and hemicellulose by determining glucose, xylose, arabinose, galactose and mannose by HPLC analysis. Conversion factors were used to convert monomer sugar concentrations into polymeric compositions in the starting materials" (Wang et al., 2011). Composition for FC is based on the mean of eight replicates. Compositions for residue materials are based on the mean of three replicates.

2.4. Autohydrolysis of fibers separated from DDGS and ground corn flour

The autohydrolysis of fiber was done using the procedure reported in Samala et al. (2012) with some modification. It was conducted in triplicates for each experimental condition, using a 750 mL Parr reactor (model 4843, Parr Instruments Co., Moline, IL, USA). Hold-times of 5, 10, 20, and 30 min at different temperatures ranging between 150 and 220 °C (in intervals of 10 °C) were used in this study. The time taken to reach 150–220 °C was 16–45 min.

The reaction mixture was filtered by gravity filtration and the filtrate was further filtered by a vacuum filtration system. The reaction mixture was filtered twice to obtain particle-free solution for HPLC analysis. The solid product was thoroughly washed with de-ionized water and dried at room temperature. The washing was collected in a bottle, labeled as liquor. The residue sample was dried in oven at 100 °C for 3 h. The residue

Table 1 – XOS contents in liquors obtained from the autohydrolysis of 10 g of corn fiber separated from DDGS. Results are means of three replicates.

Conditions		Xylo-oligosaccharides		
Hold time (min)	Temperature (°C)	Xylobiose (mg)	Xylotriose (mg)	Xylotetrose, xylopentose and xylohexose (mg)
5	150	32	124	74[a]
	160	33	142	63
	170	34[a]	193	86[a]
	180	56[a]	118[a]	57[a]
	190	119	235	191
	200	0	113	33
	210	0	7	1[a]
	220	0	1[a]	0
10	150	23	77	70
	160	30	134	27[a]
	170	63	221	150
	180	128	220	115
	190	102	216	161[a]
	200	0	88	23
	210	0	5[a]	0
	220	0	0	0
20	150	34	139	96
	160	33	151	61
	170	106[a]	192	144
	180	254	344	206
	190	275	147	58[a]
	200	0	16	8
	210	0	0	0
	220	0	0	0
30	150	30	134	102
	160	18	197	98
	170	128	243[a]	162[a]
	180	67	217	139
	190	5	80	28[a]
	200	0	21	10
	210	0	0	0
	220	0	0	0

The range of coefficients of variation for xylobiose, xylotriose, xylotetrose, xylopentose, xylohexose are 2–63%, 1–63%, 5–52%, 7–55%, 1–69% respectively.

[a] COV – ranging from 65 to 120%.

samples from some of the treatments were used for the determination of extractives, cellulose and hemicellulose content in order to verify mass balance and verify data integrity.

2.5. *Quantification of XOS, monosaccharides, and acids using HPLC*

Characterization of autohydrolysis liquor for XOS components, monosacharides, acids, furfural and hydroxymethyl furfural (HMF) by HPLC analysis of liquor was done as described by Samala et al. (2012). The instrument used was an Agilent 1200 series HPLC System device (Agilent, USA) equipped with a refractive index detector. The XOS in the liquor were analyzed using a Bio-Rad HPX 42A column at 80 °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 (Megazymes, Ireland). XOS standards with DP 7–10 were not commercially available and hence, DP 7–10 were not measured.

The monosaccharide content was determined by the HPLC unit equipped with a Bio-Rad HPX 87 P (300 mm × 7.8 mm) column at 80 °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 acidic components and sugar degradation products were using a Bio-Rad HPX 87H (300 mm × 7.8 mm) column at 80 °C and a guard column (Bio-Rad Laboratories, USA) by eluting with 0.005 M 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 HMF and furfural (Sigma–Aldrich, USA).

2.6. *Statistical analysis*

Analysis of variance (ANOVA) and Duncan's test (SAS Institute, Cary, NC) were used to compare the means of xylooligomers, monosaccharides (glucose, xylose, galactose, and arabinose), acetic acid, degradation compounds (HMF and furfural) and yields at the highest XOS production from corn fiber separated from DDGS and ground corn flour. Statistical significance level was 5% ($p < 0.05$).

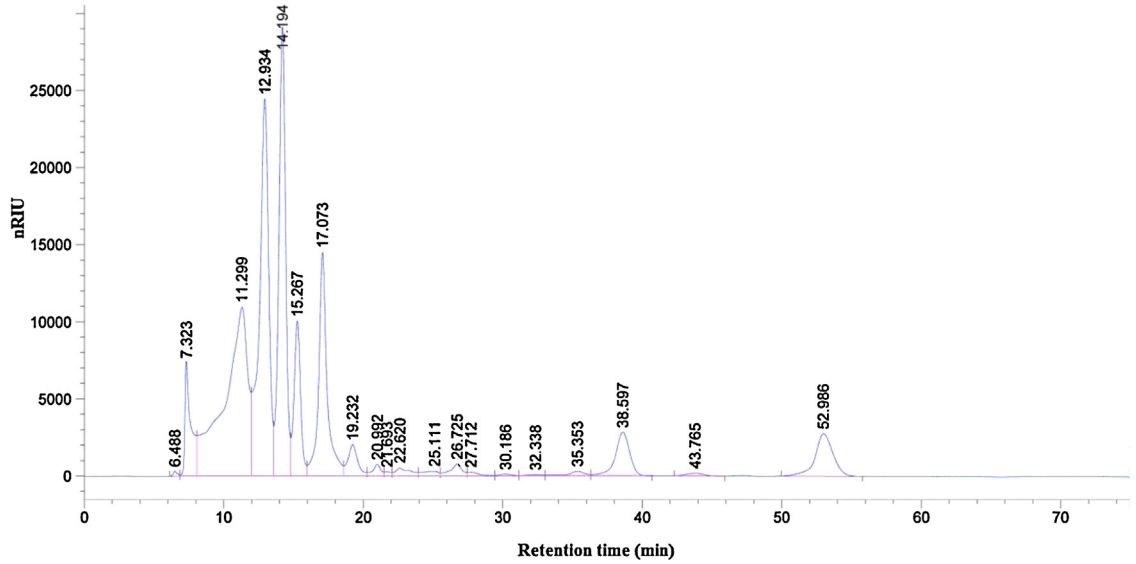

Fig. 1 – HPLC chromatogram for quantification of monosaccharides in liquor obtained by autohydrolysis of corn fiber separated from ground corn flour at 190 °C and 10 min hold-time. Using Bio-Rad HPX 87 P (300 mm × 7.8 mm) column at 80 °C by eluting with HPLC grade water. Retention times for glucose, xylose, galactose and arabinose were 12.93, 14.19, 15.26 and 17.07 min, respectively

Table 2 – XOS contents in liquors obtained from the autohydrolysis of 10 g of corn fiber separated from ground corn flour. Results are means of three replicates.

Conditions		Xylo-oligosaccharides		
Hold time (min)	Temperature (°C)	Xylobiose (mg)	Xylotriose (mg)	Xylotetrose and xylopentose (mg)
5	150	32	13	6
	160	40	19	19
	170	111	44	105
	180	66	104[a]	158
	190	460	197	133
	200	285	84[a]	41[a]
	210	67[a]	6[a]	15
	220	0	5	4
10	150	24	8	3
	160	56	27	25
	170	93	38	129
	180	76	58	243
	190	440	255	197
	200	100	11	19
	210	36	0	10
	220	0	7[a]	3
20	150	57	26	14[a]
	160	57	32	32[a]
	170	94	43	129
	180	126	54	246
	190	363	146	77[a]
	200	37	0	9
	210	33[a]	0	8[a]
	220	0	10	4[a]
30	150	59	26	17[a]
	160	67	49[a]	71[a]
	170	127	55	189
	180	204[a]	302	222
	190	248	90	19
	200	37	0	10
	210	12	0	2
	220	0	11	8[a]

Xylohexose was not detected in all of the samples. The range of coefficients of variation for xylobiose, xylotriose, xylotetrose and xylopentose are 4–67%, 3–59%, 1–61% and 3–65% respectively.

[a] COV – ranging from 65 to 120%.

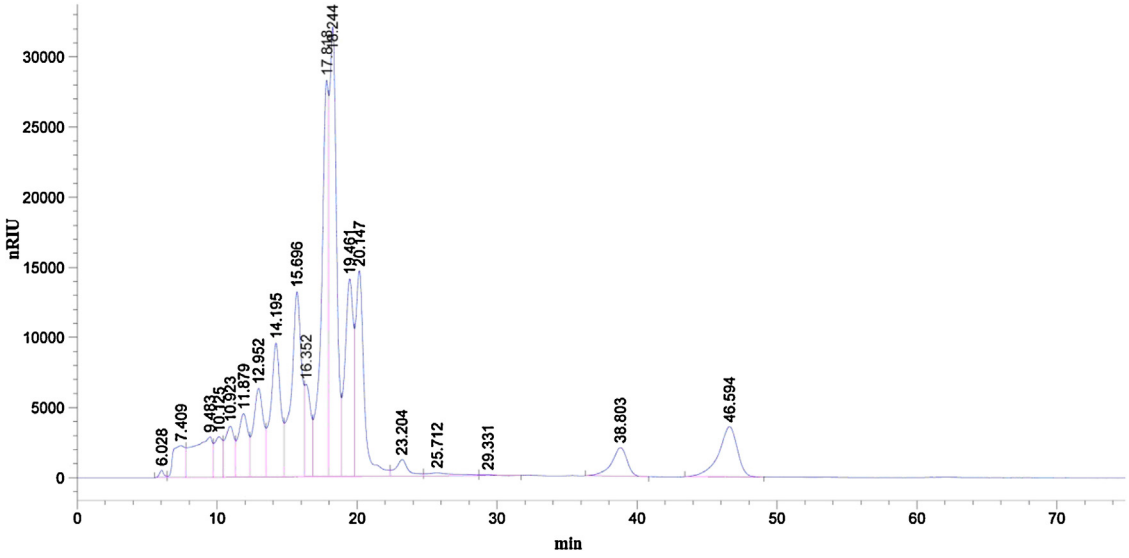

Fig. 2 – HPLC chromatogram for quantification of xylo-oligosaccharides (XOS) in liquor obtained by autohydrolysis of corn fiber separated from ground corn flour at 190 °C and 10 min hold-time. Using Bio-Rad HPX 42A column at 80 °C by eluting with HPLC grade water. Retention times for xylobiose, xylotriose, xylotetrose, and xylohexose were 14.19, 12.95, 11.97 and 10.92 min, respectively.

3. Results and discussion

3.1. *Composition of raw fiber separated from DDGS and ground corn flour*

The moisture contents of FD and FC were 12.4% and 12.7%, respectively, based on total original masses. FD had the following sugar composition: glucan 18.0%, xylan 16.8%, arabinan 8.8%, mannan 0.8%, galactan 3.0%, based on total mass of FD. FD contained 1.3% lignin, based on total mass of FD. FC had the following sugar composition: glucan 18.4%, xylan 14.3%, arabinan 6.4% and galactan 2.0%, based on total mass of FC. Glucan and xylan chains are the main polysaccharides in the raw materials used for this study. Hemicellulose content in FD and FC, comprising xylan, arabinan, galactan, and mannan was found to be 29.4% and 22.7% respectively, based on total original masses. Unaccounted components were protein and ash.

3.2. *XOS production using fiber separated from DDGS and ground corn flour*

XOS production was dependent on temperature and hold-time. The liquor obtained after autohydrolysis of FD and FC consisted mostly of a mixture of xylose oligomers with some free arabinose, glucose and galactose (Tables 1 and 2). Representative chromatograms for quantification of XOS, monosugars, acids and degradation products in the liquor, and chromatogram of the XOS standards are shown in Figs. 1–4.

For the same hold-time, XOS production increased as temperature increased till a threshold temperature and then decreased as temperature was increased further (Figs. 5 and 6).

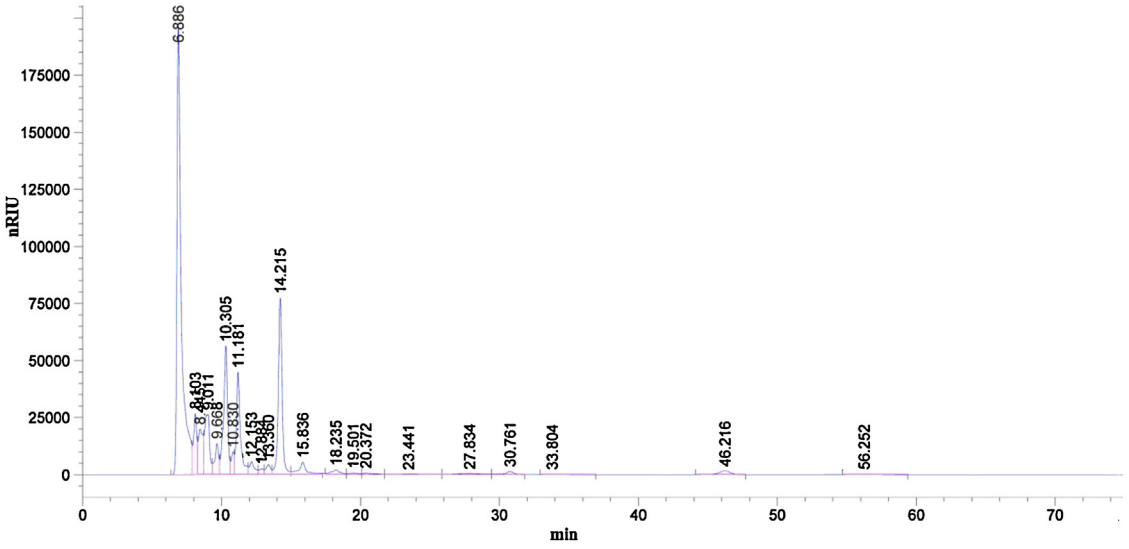

Fig. 3 – HPLC chromatogram for quantification of sugar degradation and acidic components in liquor obtained by autohydrolysis of corn fiber separated from DDGS at 180 °C and 20 min hold-time. Using Bio-Rad HPX 87H (300 mm × 7.8 mm) column at 80 °C by eluting with 0.005 M H_2SO_4. Retention times for acetic acid, HMF and furfural were 15.85, 30.66 and 46.22 min, respectively.

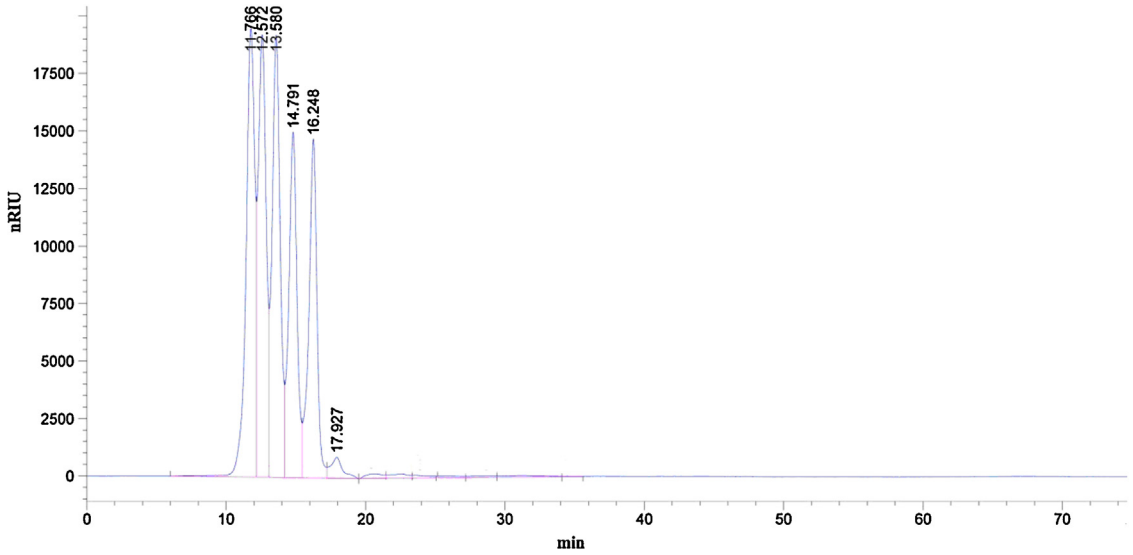

Fig. 4 – HPLC analysis of standards for quantification of xylooligosaccharides (XOS) using Bio-Rad HPX 42A column at 80 °C by eluting with HPLC grade water. Retention times for xylobiose, xylotriose, xylotetrose and xylohexose were 16.24, 14.79, 13.58, 12.57 and 11.76 min, respectively

For example, during FD autohydrolytsis at 5 min hold-time, XOS production increased from 230 mg to 544 mg as temperature was increased from 150 °C to 190 °C and then decreased to 1 mg as temperature was further increased to 220 °C (Fig. 5). As another example, during autohydrolysis of FC at 30 min hold-time, XOS production increased from 101 mg to 729 mg as temperature was increased from 150 °C to 180 °C and then decreased to 19 mg as temperature was further increased to 220 °C (Fig. 6). Thus as temperature increases, the breakdown of xylan chain into XOS increases up to a certain optimum temperature. But when temperature increases beyond the optimum level, XOS breakdown into xylose monomer and further conversion into its degradation products might be occurring causing a gradual decrease in XOS production.

The maximum production of XOS (803 mg) from FD autohydrolysis was at 180 °C temperature and 20 min hold time (Fig. 5). This was in agreement with the following previously reported results: 180 °C with 15 min hold-time (Samala et al., 2012), and 190 °C with 5 min hold-time (Carvalheiro et al., 2004). Similarly, the maximum production of XOS (892 mg) from FC autohydrolysis was at 190 °C temperature and 10 min hold time (Fig. 6). At the maximum XOS production from FD condition (180 °C and 20 min hold-time), the liquor contained xylobiose (253 mg), xylotriose (344 mg), xylotetrose (90 mg), xylopentose (70 mg), and xylohexose (46 mg) (Table 1). Similarly at the condition (190 °C and 10 min hold-time), when the production of XOS from FC was maximum, the liquor contained xylobiose (434 mg), xylotriose (255 mg), xylotetrose (130 mg), and xylohexose (67 mg) (Table 2).

The yield of XOS based on the initial xylan content in FD and FC were 54.6% and 71.5%, respectively, based on total original masses (Table 7). Xylobiose and xylotriose comprised more than 50% of the total XOS produced (Tables 1 and 2). The yields were comparable to values reported by other researchers: 52% from miscanthus (Ligero et al., 2011), 43% from barley straw (Nabarlatz et al., 2007), and 55% from bamboo (Aoyama and Seki, 1999). XOS distribution also was comparable to that reported by other researchers: XOS produced was predominantly xylobiose and xylotriose (Aoyama and Seki, 1999; Nabarlatz et al., 2007). Ligero et al. (2011) did not determine

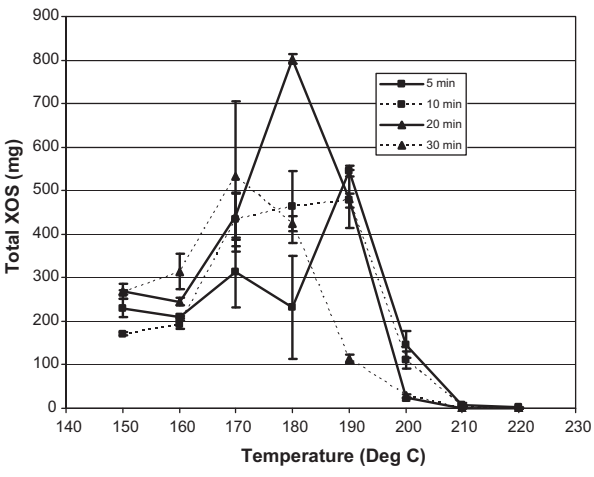

Fig. 5 – Total XOS (mg) produced from autohydrolysis of 10 g of corn fiber separated from DDGS, at different temperatures with hold-times of 5, 10, 20 and 30 min.

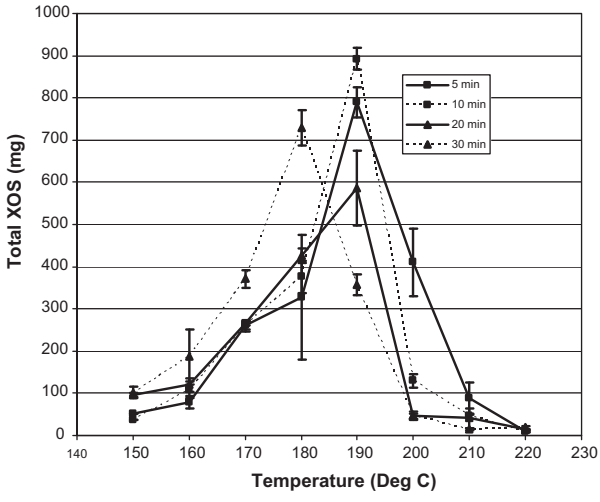

Fig. 6 – Total XOS (mg) produced from autohydrolysis of 10 g of corn fiber separated from ground corn flour, at different temperatures with hold-times of 5, 10, 20 and 30 min.

Table 3 – Monosaccharides, acid and degradation compounds contents in liquors obtained from autohydrolysis of 10 g of corn fiber separated from DDGS. Results are means of three replicates.

Conditions		Monosaccharides					Degradation products		Acids
Hold time (min)	Temperature (°C)	Glucose (mg)	Xylose (mg)	Galactose (mg)	Arabinose (mg)	Total (mg)	HMF (mg)	Furfural (mg)	Acetic acid (mg)
5	150	147	85	28	193	453	0	0	896
	160	182	121	52	270	626	0	0	901
	170	301	220	122	303	947	0	0	1396
	180	183[a]	133[a]	65[a]	241[a]	623	0	0	795
	190	264	349	168	266	1047	46	39	1412
	200	223	130	89	109	550	163	210	2029
	210	85[a]	12	21	16[a]	135	165	85	1408
	220	14	4[a]	10	21	49	239	102	2485
10	150	110	74	20	137	342	0	0	1011
	160	184	123	53	267	627	0	0	1021
	170	351	269	150	300	1069	0	0	1244
	180	348	253	135	378	1114	0	0	1386
	190	336	342	163	237	1078	48	23	1208
	200	152[a]	56[a]	55[a]	72	335	185	84	2091
	210	92[a]	33	35[a]	38	198	186	94	1591
	220	14	3[a]	9	18	43	278	144	2839
20	150	161	102	34	236	533	0	0	1216
	160	244	173	87	330	834	0	0	1081
	170	316	280	147	224	967	0	0	1502
	180	493	378	204	469	1544	0	0	1724
	190	266	191	118	108[a]	684	147	62	1883
	200	76	12	26	35	149	213	45	1745
	210	16	6	9	28	56	208	63	2083
	220	8	4	9	3	24	160	53	2403
30	150	185	122	44	269	621	0	0	1043
	160	310	229	130	374	1042	0	0	1290
	170	391	382	194	256	1223	0	0	1107
	180	318	254	138	270	979	0	0	1367
	190	196	98	79	29[a]	402	166	51	1830
	200	104	16	34	23	176	175	93	1365
	210	17	8	10	17[a]	51	202	46	2112
	220	7	4	8	16	35	128	56	2625

The range of coefficients of variation for glucose, glucose, xylose, arabinose, galactose were 5–64%, 2–57%, 3–61%, 4–64%, respectively.

[a] COV – 65–115%.

Table 4 – Monosaccharides, acid and degradation compounds contents in liquors obtained from the autohydrolysis of 10 g of corn fiber separated from ground corn flour. Results are means of three replicates.

Conditions		Monosaccharides					Degradation products		Acids
Hold time (min)	Temperature (°C)	Glucose (mg)	Xylose (mg)	Galactose (mg)	Arabinose (mg)	Total (mg)	HMF (mg)	Furfural (mg)	Acetic acid (mg)
5	150	132	98	34	241	506	0	0	0
	160	203	143	50	323	719	0	0	0
	170	395	315	131	485	1326	0	0	112
	180	421	401	160	374	1356	7	18	214
	190	762	634	225	381	2003	112	97	370
	200	788	502	194	310	1793	388	198	259
	210	563	184	112	148	1007	318	136	271
	220	112	14	29	30	186	106	129	245
10	150	112	63	22	163	360	0	0	0
	160	247	181	65	382	876	0	0	0
	170	418	343	145	483	1389	0	0	194
	180	526	497	208	480	1711	24	12	189
	190	712	640	234	410	1996	89	89	361
	200	742	287	151	209	1389	298	176	227
	210	472	90	90	105	757	378	152	247
	220	54	18	19	24	115	310	135	361
20	150	149	104	39	232	524	0	0	0
	160	269	199	73	406	946	0	0	0
	170	421	343	146	483	1393	0	0	210
	180	541	526	214	475	1755	24	21	300
	190	776	561	206	338	1882	140	83	207
	200	471	113	92	116	792	227	117	301
	210	343	105[a]	70	89[a]	607	315	118	208
	220	27	14	17	21	80	427	146	472
30	150	227	153	53	351	785	0	0	0
	160	291	229	90	398	1009	0	0	0
	170	438	394	166	433	1431	0	0	215
	180	598	609	225	420	1852	54	47	308
	190	882	480	196	297	1855	180	91	303
	200	495	92	95	110	792	241	154	373
	210	105	13	25	24	167	422	109	257
	220	29	16	20	23	89	461	182	628

The range of coefficients of variation for glucose, glucose, xylose, arabinose, galactose were 2–57%, 1–57%, 1–64%, 1–65%, respectively.

[a] COV – ranging from 65 to 135%.

Table 5 – Mass balance of sugars for corn fiber separated from DDGS at a few selected conditions.

Conditions	Material	Glucose	Xylose	Galactose	Arabinose	Total
None	Input (mg)	1577	1472	263	771	4082
150 °C, 5 min	Liquor (mg)	129	305	25	169	627
	Residue (mg)	977	917	80	389	2362
	% Unaccounted	30	17	60	28	27
150 °C, 30 min	Liquor (mg)	162	373	39	235	809
	Residue (mg)	1124	533	44	177	1879
	% Unaccounted	18	38	68	46	34
160 °C, 5 min	Liquor (mg)	160	344	46	237	786
	Residue (mg)	1189	621	48	201	2059
	% Unaccounted	14	34	64	43	30
170 °C, 5 min	Liquor (mg)	264	505	107	266	1142
	Residue (mg)	1117	155	0	23	1295
	% Unaccounted	12	55	59	63	40
180 °C, 5 min	Liquor (mg)	161	348	57	211	776
	Residue (mg)	1117	46	0	0	1163
	% Unaccounted	19	73	78	73	52
180 °C, 10 min	Liquor (mg)	305	685	118	331	1439
	Residue (mg)	1131	36	0	0	1167
	% Unaccounted	9	51	55	57	36
180 °C, 20 min	Liquor (mg)	432	1134	179	411	2155
	Residue (mg)	1012	31	0	0	1043
	% Unaccounted	8	21	32	47	22
180 °C, 30 min	Liquor (mg)	278	646	121	237	1282
	Residue (mg)	1008	23	0	0	1032
	% Unaccounted	18	55	54	69	43

the distribution of XOS. The maximum XOS yields obtained from original feedstock in this study (8.9% from FC and 8.0% from FD) were comparable to the maximum XOS yield reported by earlier autohydrolysis studies (Table 7, Samala et al., 2012). The maximum XOS yield from brewery's spent grain (BSG) and almond shells were 14.1% (Carvalheiro et al., 2004) and 15.7% (Nabarlatz et al., 2007), respectively, based on total original masses.

Table 6 – Mass balance of sugars for corn fiber separated from ground corn flour at a few selected conditions.

Conditions	Material	Glucose	Xylose	Galactose	Arabinose	Total
None	Input (mg)	1868	1432	192	646	4138
150 °C, 5 min	Liquor (mg)	115	137	29	211	493
	Residue (mg)	1508	872	100	476	2956
	% Unaccounted	13	29	33	-6	17
150 °C, 30 min	Liquor (mg)	198	235	47	306	786
	Residue (mg)	757	964	335	31	2087
	% Unaccounted	49	16	-99	48	31
160 °C, 5 min	Liquor (mg)	177	204	44	282	707
	Residue (mg)	1342	494	39	217	2092
	% Unaccounted	19	51	57	23	32
170 °C, 5 min	Liquor (mg)	345	535	115	423	1417
	Residue (mg)	473	1043	123	0	1640
	% Unaccounted	56	-10	-24	34	26
180 °C, 5 min	Liquor (mg)	368	678	139	327	1512
	Residue (mg)	1321	59	0	9	1389
	% Unaccounted	10	49	27	48	30
180 °C, 10 min	Liquor (mg)	459	811	181	419	1870
	Residue (mg)	668	116	0	0	784
	% Unaccounted	40	35	6	35	36
180 °C, 20 min	Liquor (mg)	472	884	187	415	1958
	Residue (mg)	1258	57	0	0	1316
	% Unaccounted	7	34	3	36	21
180 °C, 30 min	Liquor (mg)	522	1260	196	367	2346
	Residue (mg)	1302	0	0	0	1302
	% Unaccounted	2	12	-2	43	12

Table 7 – Comparison of xylooligomers content (mg) obtained from the autohydrolysis of 10 g of corn fiber separated from DDGS and ground corn flour at maximum production condition. Results are means of three replicates. Means in the same column for each xylooligomer followed by same letter are not significantly different ($p < 0.05$).

Raw material	Condition	Xylobiose (mg)	Xylotriose (mg)	Xylotetrose, xylopentose and xylohexose (mg)	Total XOS (mg)	Yield % of total	Yield %, based on xylan
FD	180 °C, 20 min	254[b]	344[a]	206[a]	803[b]	8.0[b]	54.6[b]
FC	190 °C, 10 min	440[a]	255[b]	197[a]	892[a]	8.9[a]	71.5[a]

Table 8 – Comparison of monosugar, degradation compound, and acid contents (mg) in liquors obtained from the autohydrolysis of 10 g of corn fiber separated from DDGS and ground corn flour at maximum XOS production condition. Results are means of three replicates. Means in the same column followed by same letter are not significantly different ($p < 0.05$). Glu, Xyl, Gal, Ara–Glucose, Xylose, Galactose, Arabinose respectively.

Raw material	Condition	Glu (mg)	Xyl (mg)	Gal (mg)	Ara (mg)	HMF (mg)	Furfural (mg)	Acetic acid (mg)	Total mono (mg)
FD	180 °C, 20 min	493[b]	378[b]	204[a]	469[a]	0[b]	0[a]	1724[a]	1544[a]
FC	190 °C, 10 min	712[a]	640[a]	234[a]	410[a]	89[a]	89[a]	361[b]	1996[a]

3.3. *Comparison of XOS production from fibers separated from DDGS and ground corn flour*

The conditions for maximum XOS production from FD and FC were 180 °C with 20 min hold-time and 190 °C with 10 min hold-time, respectively (Table 7). XOS yield from FC, based on the initial xylan used, was higher (71.5%) than from FD (54.6%) because initial xylan content of FC was lower than FD. XOS yield based on the total initial material also was higher for FC (8.9%) than for FD (8.0%). Thus, fiber separated from ground corn flour resulted in higher XOS yield than fiber separated from DDGS (Table 7).

There was no significant difference in the total monosaccharides content in liquors from FC and FD at the condition of maximum XOS production. Acetic acid content in liquor from FD was higher than the liquor from FC. HMF and furfural content were present in trace quantities (total 89 mg) in liquor from FC, while they were absent in the liquor from FD. The absence or presence in trace quantities of HMF and furfural in the liquor, at maximum XOS production conditions, would be beneficial when used as prebiotics.

3.4. *Monosaccharides, acids, furfural and hydroxymethylfurfural (HMF)*

At the same hold-time, when the temperature increased, monosaccharides content in liquor increased until a threshold temperature reached and but decreased on further increase of temperature (Tables 3 and 4). As for an example, in case of FC, at 10 min hold-time, the total monosaccharides content increased from 506 mg to 2003 mg as temperature was increased from 150 °C to 190 °C but decreased to 186 mg as the temperature was further increased to 220 °C (Table 4). The increase in monosaccharides content with increase in temperature can be attributed to the breakdown of carbohydrates into monosaccharides and the decrease in monosaccharides content can be attributed to the conversion of the monosaccharides into other compounds (breakdown products). Pentose degradation leads to formation of furfural, whereas hexose degradation leads to formation of HMF. Such degradation of arabinose at higher temperatures has been reported by Kootstra et al. (2009).

For the same hold-time, the production of acetic acid was found to be increased on the increase of temperature (Tables 3 and 4). Ligero et al. (2011), and Nabarlatz et al. (2007) also reported higher acetic acid contents at higher temperatures. Acetic acid formation was due to the breakdown of acetyl groups attached to the xylan chain. At similar autohydrolysis conditions, acetic acid production was higher from FD than FC (Tables 3, 4 and 8). A higher acetic acid production from FD might be due to acetyl groups in the xylan chain of DDGS being partially loosened during the thermal treatment used in the jet cooking and DDGS drying stages of fuel ethanol production.

For the same hold-time, HMF and furfural contents in liquor increased on the increase of temperature until a threshold temperature reached and then stayed constant or increased on further temperature increase (Tables 3 and 4). Ligero et al. (2011), and Nabarlatz et al. (2007) also reported higher HMF and furfural contents at higher temperatures. It has been reported by several investigators that such HMF and furfural formation is due to the degradation of monosaccharides (Kootstra et al., 2009; Carvalheiro et al., 2004).

3.5. *Mass balance for verification of data integrity*

Component-wise and overall mass balances at selected conditions are reported in Tables 5 and 6. Mass balance calculation is based on the soluble sugars (monomers and oligomers as monomeric equivalents) in the liquor solution and residue obtained after the autohydrolysis.

It was found that the unaccounted total monosugars was 17–52% and the range of component-wise unaccounted material for glucose, xylose, galactose and arabinose were 2–56%, 12–73%, 3–99% and 23–73%, respectively, based on input components (Tables 5 and 6). The mass balance error was higher for galactose, which can be due to the low quantities of galactose in the materials. The mass balance errors were within acceptable limits in the most of the conditions, it can be concluded that the data integrity was good.

4. Conclusions

The conditions for maximum XOS production from fibers separated from DDGS and ground corn flour were 180 °C with 20 min hold-time and 190 °C with 10 min hold-time respectively. Based on the initial xylan content, the fiber separated from ground corn flour (FC) resulted in higher yield of XOS (71.5%) than the fiber separated from DDGS (FD) (54.6%) at the maximum XOS production conditions. Based on total initial

material also, XOS yield was higher for FC (8.9%) than for FD (8.0%). Thus, fiber separated from ground corn flour would be a better feedstock for production of XOS than fiber separated from DDGS. The absence or presence in trace quantities of degradation products (HMF and furfural) in the liquor, at maximum XOS production conditions, would be beneficial when used as prebiotics. Thus, autohydrolysis of corn fiber can be used to produce XOS, a high-value product that has application as prebiotics.

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