ORIGINAL RESEARCH PAPER

Home Science

ALKALINE EXTRACTION OF XYLAN FROM AGRO WASTE AND DETERMINING XYLOOLIGOSACCHARIDE (XOS) USING ENZYMATIC HYDROLYSIS.

KEY WORDS:
Xylooligosaccharide, Crude xylan, Xylanase, Enzymatic hydrolysis.

Abnita Thakuria PhD Research Scholar, Department of Food and Nutrition, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat (India), 390002.

Mini Sheth * Professor, Department of Food and Nutrition, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat (India), 390002. *Corresponding Author

ABSTRACT

In India, an estimated amount of 120-150 million tons of agro wastes remains as surplus per year which can be potentially used to produce various value added products like bio fuels, animal feeds, enzymes etc. These can be utilized to manufacture several value based products including Xylooligosaccharide (XOS) which may exhibit prebiotic effect when consumed regularly. The objective of this study was to quantify the XOS present in selected agro waste namely corncob, green pea shells, raw green banana, and orange peels. Alkaline extraction method was used to determine Xylan from these agro wastes and further XOS was extracted using enzymatic hydrolysis and was quantified using HPLC. The XOS yielded from crude xylan of corncob, orange peels, raw green banana, and green pea shells were 91.11% (1.8g), 92.36% (1.41g), 95.05% (1.01g), and 92.40% (0.79g), respectively at ($p \leq 0.01$). Hence, the study revealed a possibility of extracting XOS from different agro waste.

1. INTRODUCTION

The residues from the growing and processing of raw agricultural products such as fruits, vegetables, meat, poultry, dairy products and crops are defined as agricultural wastes. Agricultural wastes can be in the form of solid, liquid or slurries depending on the nature of agricultural activities (C.N. Foster, 2015). In India, 620 million tons of agricultural and agro-industrial residues are being generated approximately (Singh et al, 2017). Around seventy percent of these agricultural wastes are used as fodder, fuel for domestic and industrial sectors etc. (MNRE in association with Indian Institute of Science, Bangalore, 2014). Therefore, an estimated amount of 120-150 million tons of agro wastes or residues remains as a surplus per year and can potentially be used to produce various value added products like bio fuels, animal feeds, chemicals, enzymes etc. (Saha, 2003; Goldman, 2009). There is a huge scope for the value addition and utilization of these agricultural waste or residues for food applications such as production of XOS, xylitol and xylose (Aachary and Prapulla, 2009). Xylooligosaccharides (XOS) are sugar oligomers containing 2-7 xylose monomeric units linked through –β-(1-4)- linkages produced from xylan hydrolysis and has characteristics of a prebiotic. XOS when consumed as a part of daily diet promotes the growth of probiotic organisms such as *Bifidobacteria and Lactobacillus* in the colon, has great potential for use in medicines, food and health products (Moure *et al. 2006;* Gupta *et al. 2016)*.

XOS can be produced by enzymatic, chemo-enzymatic, partial hydrolysis of xylan from various sources such as barley hulls, rice hulls, corn cobs, peanut pods, sugarcane baggase, wheat straw, cotton stalks, orange peels, mango peels etc.(Moure *et al. 2006;* Gupta *et al. 2016*). This technology can be transferred to the fruit and vegetable, nuts and oilseeds industries etc. for production of XOS from the agricultural wastes produced from their industries and thereby add to the country's economic growth by making it available in the market nationally and also at Global level for export. Hence, the primary objective of the study was to determine the extent to which XOS can be extracted from the selected agro waste so that it can be used as a value added product in health and diseases. The agricultural wastes selected in this study to be explored for the extraction of XOS were corncobs, orange peels, raw green banana peels and green pea shells. From the several methods of XOS extraction, enzymatic hydrolysis was selected as the method of extraction in this study as enzymatic hydrolysis with xylanase does not produce any toxic by-products unlike other methods (Gupta et al. 2015).

2. MATERIALS AND METHOD

The alkaline extraction of xylan and HPLC analysis of XOS was carried out at Dr. Nagar's Laboratories Ltd., Gorwa, Vadodara, Gujarat, India.

2.1. Sample collection

Oranges, Raw green bananas and green peas were collected from Anand Agricultural University Model Farm, Vadodara, Gujarat and the varietal names were identified with the help of the agricultural officer. Corn cob powder was procured from Rahi Industries, Mehsana, Gujarat. The varietal names of the samples were Orange (Mosambi), Raw green banana (G9), Green pea (Prakash) and Corn cob (SUGAR 75). All the samples were procured in April, 2017.

2.2. Sample preparation

The oranges and raw green bananas were peeled and green peas were de shelled followed by washing 4-5 times under running water to remove the dust and dirt particles. These were then dried under the fan for 2h to remove the surface moisture. The samples were then weighed using a digital electronic balance having an accuracy of 0.01g. 2 kg of fresh green peas, 3 kg of raw green bananas and 2 kg of oranges yielded 630 g of pea shells, 350 g of banana peels, 350 g of orange peels, respectively. The orange and green banana peels were dried at 65°C-70°C for 12h in a hot air oven. The pea shells were dried at 65°C for 8h. The samples were weighed after every 10 min until the drying rate became constant for 1h. The samples were allowed to cool down to room temperature and were grounded to fine powder and sieved. The weight of the powdered samples for orange peels, raw green banana peels and green pea shells were 115 g, 74 g and 121 g, respectively. The powdered forms of the samples were stored in airtight containers.

2.3. Alkaline extraction of xylan from corncob powder, orange peel powder, green banana peel powder and green pea shell powder

Sodium hydroxide solution (4% w/v) was taken in 5000 mL round bottom flask (RBF). Each of the four samples were weighed up to 60 g and added to the solution in the four different RBF followed by thorough mixing and steaming at 100°C for 5h. The solutions were then allowed to cool at 25°C and were centrifuged at 6000 rpm for 20 min. These were then allowed to settle for the separation for 10 min. The supernatant layer was separated and acidified with 1N HCl solution (710 mL) to pH 5.0. Ethanol (3000 mL) was added in order to precipitate the xylan. Using a Buchner funnel under vacuum the precipitated xylan was filtered. The crude xylan was allowed to dry in an air tray dryer for 12h. Once the crude xylan dried completely, they were sieved using a 100 Mesh sieve. The true yield of the xylan was calculated using the formula (1) shown in fig. 2.3.1.

$$\text{True yield (\%)} = \frac{\text{Dry weight of extracted xylan (g)}}{\text{Weight of the sample (g)}} \times 100 \quad (1)$$

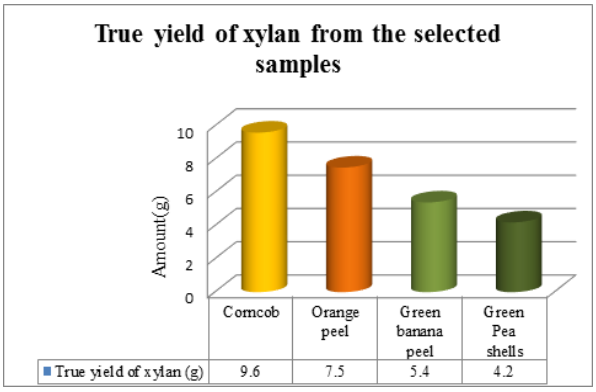

Fig. 2.3.1: True yield of xylan from corncob, orange peel, green banana peel and green pea shells.

Each of the sample's xylan was further divided into four equal portions for enzymatic hydrolysis.

Corncob (S_1) = 9.60/4 = 2.40 g, Orange peel (S_2) = 7.50/4 = 1.87 g, Green banana peel (S_3) = 5.40/4 = 1.35 g, Pea shell (S_4) = 4.20/4 = 1.05 g.

2.4. Enzymatic hydrolysis of Xylan

All the four portions of each sample were hydrolyzed at different incubation time. In a 500 mL Erlenmeyer flask 2.4 g xylan derived from S1 was added to 250 mL deionized water (DI water). 2.0% xylanase enzyme procured from Siga, India was added to the flask. A sufficient quantity of buffer (Ammonium hydrogen sulphate) was added to the solution to bring it to pH 7.0. The solution was stirred properly and incubated at 40 C for 4h, 6h, 8h and 12h, respectively. The aliquots were taken at the respective time intervals (4h, 6h, 8h, and 12h) and chilled thoroughly using ice. The aliquots were then centrifuged at 6000 rpm for 20 min. The supernatant was then separated and filtered through a sintered funnel. The supernatant layer has crude XOS.

2.5. Purification of XOS

There are several treatments for the refining of XOS such as solvent extraction and precipitation, chromatographic separation for the purification of XOS, membrane technology for the purification of XOS, adsorption etc. Adsorption has been used intending either the separation of oligosaccharides from monosaccharides (Sanz et al. 2005, Vazquez et al. 2000) or to remove the undesirable compounds (Kokubo et al. 2004, Yuan et al. 2004). Montane et al. 2006 used activated carbons for the purification of XOS produced by auto hydrolysis of almond shells.

In the present study activated charcoal was used which comes under adsorption for purification of XOS.

A vertical glass column having diameter 26mm and length 450mm was used and 28gs of activated charcoal was added into it. The activated charcoal in the column was washed with DI (deionized) water. The DI water was then poured in the column to make a homologous bed layer. The supernatant layer was preloaded in the activated charcoal bed in the column. The mobile phase was run for 6h upon increasing ethanol (30%): DI water ratio approximately until the pure form of XOS was obtained. The solvent was distilled at 50 C on Rota evaporator under vacuum. The pure XOS contains xylose, xylobiose, xylotriose, xylotetrose , xylopentose etc. The XOS obtained was dried at 50 C using air tray dryer.

2.6. Assay of XOS

The XOS content of xylan samples were determined by high-performance liquid chromatography (HPLC) having an Inertsil NH2 column (250 × 4.5 mm) and refractive index (RI) detector. 20µl of the sample was injected into the column, where XOS was eluted using a mobile phase of (Acetonitrile) ACN:H2O (70:30, v/v) at the flow rate of 1.0 mL/min for 30 min. In the present study, alkali extracted xylan of the selected agro wastes were hydrolyzed by commercial xylanase enzyme. The effect of temperature, enzyme dose, pH and reaction time on the production of XOS was determined. Levels of pure XOS was determined from XOS obtained from the 12h incubation period batch using HPLC. All the analysis was carried out in triplicates. As the concentration of standard XOS was 1g/10mL. Therefore 20µl of the standard contains 2mg of XOS. The areas covered by the peaks were considered and mean of the areas were obtained. The mean area of XOS standard was 4196267.66 which contained 2mg of XOS. In the present study, the concentration of XOS from corncob, green banana peel, orange peel and green pea shells were calculated using the formula:

$$\text{Concentration of XOS} = \frac{\text{Average area of the sample concerned}}{\text{Average area of the standard XOS}} \times 100 \quad (2)$$

2.7. Statistical analysis

The HPLC analysis of XOS from all the agro waste samples and XOS standard were conducted in triplicates for each of the samples. The effect of incubation period on the yield of XOS was conducted at 4h, 6h, 8h and 12h. Data were collected and analyzed by using one-way analysis of variance (ANOVA). The significant differences between tests were set at $p \leq 0.05$. All statistical analyses were performed using Microsoft office excel 2007.

1. Results and discussion

1.1. Determination of Xylan in selected agro waste

The second most available biopolymer of the plant kingdom is Xylan and the major form of hemicelluloses found in agricultural by-products. Xylan has a wide variety of applications in diversified fields which have not been exploited so far (Samanta et al, 2015). In the present study, different levels of XOS yield were determined from xylan of the four selected agricultural wastes using 4% sodium hydroxide (NaOH). During alkaline extraction, steam application is suggested to enhance the yield of xylan, therefore, in this study the broth was steamed at 100 C for 5h. The crude xylan yield from 60 g of each of the samples was 9.60 g (16.0%), 5.40 g (9.0%), 7.50 g (12.5%) and 4.20 g (7.0%), respectively.

Samanta et al, 2012, attempted to extract the xylan from *S. nervosum* grass with incremental levels (2%, 4%, 8% and 12%) of both sodium hydroxide (NaOH) and potassium hydroxide (KOH). They further investigated the effect of different alkali on the recovery of xylan from particular grass under overnight incubation at room temperature (16h, 25 C) or autoclaving (121 C, 15 lbs, 45 min). They reported that during overnight incubation at room temperature, the incremental levels of either potassium hydroxide or sodium hydroxide resulted in increase in true recovery of xylan from 2.47% to 16.52% and 3.75% to 25.12% of original biomass, respectively. 4% KOH and NaOH yielded 6.28% and 8.35% xylan, respectively during overnight incubation. A similar study by Yang et al, 2007 reported that when corncob, bagasse, wheat bran and peanut shell was exposed to 4% (w/v) NaOH and steamed at 100 °C for 3h, xylan yielded from these samples were 12.5%, 15.7% 18.5% and 3.5%, respectively.

Therefore, further scope of investigation exists in the similar line with several combinations such as pH, temperature, incubation time and enzyme dosage to find out the best method that gives highest yield (Table. 3.1).

Table3.1: XOS yield by enzymatic hydrolysis of Xylan at different incubation and pH - 5.5 at 40°C.

Incubation time	Corncob(g)	Banana peel(g)	Orange peel(g)	Green pea shells(g)
4h	1.44	0.81	1.13	0.63
6h	1.58	0.89	1.24	0.69
8h	1.70	0.96	1.33	0.75
12h	1.80	1.01	1.41	0.79
F value	5.98	5.98	5.98	5.98
'p' value	0.013**	0.008**	0.010**	0.007**

Significant at *p≤0.05, **p≤0.01, *** p≤0.001

1.1. Enzymatic hydrolysis of Xylan

There are several processes of production of XOS from xylan. Enzymatic hydrolysis is preferred over others as it neither generates toxic compounds nor requires special equipment (Samanta et al. 2012). Production of XOS from various sources of xylan such as corncob, birchwood, wheat bran, tobacco stalk etc. using commercial xylanases have been reported by many researchers. Fewer attempts were made for production of XOS using indigenously produced xylanases. A study was conducted in which xylanase was produced using a low cost technique with wheat bran as a substrate and anaerobically treated distillery spent wash as the moistening agent by *A. foetidus* (Chapla et al. 2012). Another study was conducted to produce XOS using orange peels as substrate and the source of enzyme was *Aspergillus niger* (Gupta et al. 2015). In another study, 3 commercial xylanase preparations (Rapidase Pomaliq from Gist-Brocades, Clarex ML from Generor and Validase from Valley Research) were evaluated as a sole enzyme source for the enzymatic production of pentoses from the hemocellulose fraction of corn husks and corncobs. The results indicated that Rapidase Pomaliq, an enzyme from Aspergillus niger and Trichoderma resei, could serve as the sole enzyme source for the production of pentoses and XOS from corn residues (Achary et al. 2011).

In the present study, the extracted xylan was further divided into four equal portions for enzymatic hydrolysis to obtain XOS. Commercial xylanase enzyme (2.0%) procured from Sigma, India was used to hydrolyze xylan. They were exposed to different incubation time such as 4h, 6h, 8h and 12 h with pH 5.5 at 40°C. A significant rise in the yield of XOS was observed as the incubation time increased from 4h-12h ($p \leq 0.01$) for all the four products (Table 3.1). The present study revealed that pure XOS obtained from the xylan corncob, orange peels, raw green banana, and green pea shells were 91.11% (1.8g), 92.36% (1.41g), 95.05% (1.01g), and 92.40% (0.79g), respectively at ($p \leq 0.01$) with an optimal condition of 12h incubation time, pH 5.4 at 40°C. Although all the four samples yielded high amounts of XOS, orange peels yielded the highest amount of XOS from xylan followed by banana peels.

Akpinar et al. 2007, found that cotton stalk, which had no economical value, could be converted by enzymatic hydrolysis to a more valuable XOS product. 24 h of hydrolysis yielded 53% XOS at 40°C. Another study conducted by Yang et al. 2007 revealed the production of XOS from various xylan obtained from corncob, bagasse, wheat bran and peanut shell by extracellular xylanases from *Thermobifida fusca* NTU22 was 29.5%, 23.7%, 7.6% and 10.1%, respectively.

1.2. Concentration of XOS among all the agro waste samples

A study conducted by Gupta et al. 2014-2015 reported that the amount of XOS in freeze dried samples of sweet lime peel and orange peel (retentate and permeate) was 190 mg/mL and 333 mg/mL, 146 mg/mL and 558 mg/mL, respectively. Therefore, it was concluded that orange peel is the best out of the two substrates for producing XOS.

Concentration of XOS was found to be highest for corncob followed by orange peels, green banana peels and green pea shells (Table 3.3). Another study conducted by Samanta et al. 2015 reported that they found a total concentration of XOS derived from corncob (excluding xylose) varied from 1.19 to 1.69 mg/mL, depending on pH, temperature of reaction, dose of enzyme and duration of hydrolysis. Whereas, the present study resulted into higher concentration of XOS derived from corncob (79.41mg/mL), orange peels (74.73 mg/mL), green banana peels (73.50 mg/mL) and green pea shells (71.94 mg/mL)

Table 3.3: Comparative results of the concentration of XOS among all the agro waste samples

Sl. No.	Sample name	Concentration of XOS (mg/mL)
1.	Standard XOS	100
2.	Corncob's XOS	79.41
3.	Green banana peel's XOS	73.50
4.	Orange peel's XOS	74.73
5.	Green pea shell's XOS	71.94

4. CONCLUSION

This study has successfully established that agro wastes like corncobs, orange peels, green banana peels and green pea shells can be utilized as a source to manufacture XOS and benefit human health. Several researchers have explored corncobs, sugarcane baggase, rice husk, wheat husk etc. in order to extract xylooligosaccharide. Very few studies have been conducted on orange peels. The present study for the first time has tried to explore green pea shells and green banana peels to check the presence of XOS. Several studies on corncob and other products have reported different levels of XOS content. There might be a correlation between varietal differences and XOS content. Hence, there is a great scope to explore several varieties of agricultural products. This methodology needs to be explored further and refined by seeing the difference in yield of XOS with different levels of pH, temperature enzyme dosage and different varieties. It will allow optimizing the conditions (pH, temperature, incubation time and enzyme dosage) in order to obtain maximum yield of XOS in many more agro waste. The method can be further modified for industrial set up and manufacture XOS out of agro waste at commercial level in order to benefit human health and also add to the country's economic growth by making it available in the market nationally and also at Global level for export.

5. Acknowledgement

This study was partially funded by The M.S. University of Baroda under the University Research Scholarship.

6. Conflicts of interest

The authors do not have any conflicts of interest to declare.

REFERENCES

1. Aachary, A.A. and Prapulla S.G. (2009). Value addition to corncob: Production and characterization of xylooligosaccharides from alkali pretreated lignin-saccharide complex using Aspergillus oryzae MTCC 5154. Bioresource Technology. 100 (2), pp. 991-995.
2. Aachary, A.A. and Prapulla, S.G. (2011). Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications. Comprehensive Reviews in Food Science and Food Safety. 10(1), pp. 2-16.
3. Abdullah F. (2014). Production of Energy from Agricultural Waste. Press Information Bureau. Government of India. Ministry of New and Renewable Energy (MNRE). Retrieved from http://pib.nic.in/newsite/PrintRelease.aspx?relid=104199 on 02/03/2018.
4. Akpinar O, Ozlem Ak, Kavas A, Bakir U and Yilmaz L. (2007). Enzymatic Production of Xylooligosaccharides from Cotton Stalks. Journal of Agricultural and Food Chemistry. 55(14), pp. 5544-5551.
5. Chapla D, Pandit P and Shah A. (2012). Production of xylooligosaccharides from corncob xylan by fungal xylanase and their utilization by probiotics. Bioresource Technology. 115, pp. 215-221.
6. Foster, C.N. (2015), Agricultural Wastes: Characteristics, Types and Management. Nova Science Publishers. ISBN: 978-1- 63482-359-3.
7. Goldman (2009). Methods for optimizing enzymatic hydrolysis of xylan to improve xylooligosaccharides, MMG 445. Basic Biotechnology., 5, pp. 31-36.
8. Gupta P.K., Agrawal P and Hegde P. (2014-2015). Extraction of Xyloloigosacch arides by using Aspergillus niger from orange wastes. International Journal of PharmTech Research. 7(3), pp. 488-496. ISSN: 0974-4304.
9. Gupta P. K. , Agrawal P., Hegde P., Shankarnarayan N.,Vidyashree S., Singh S. A. and Ahuja S. (2016). Xylooligosaccharide –A valuable material from waste to taste: A review. Journal of Environmental Research and Development. 10(3). pp. 555-563.
10. Kokubo I, Ikemizu S. (2004). Histamine-release inhibitors containing xylooligosacc harides. Japan Patent JP 2004059481:2004.
11. Montanne D, Nabarlatz D, Martorell A, Torne- Fernandez V & Fierro V (2006). Removal of lignin and associated impurities from xylooligosaccharides by activated carbon adsorption. Industrial and engineering Chemical Research. 45(7), pp. 2294-2302.
12. Moure, A., Gullon, P., Dominguez, H., & Parajo, J. C. (2006). Advances in the manufacture, purification and applications of xylooligosaccharides as food additives and nutraceuticals. Process Biochemistry. 41(9). pp.1913–1923.
13. Saha, B.C. (2003). Hemicellulose bioconversion. Journal of Industrial Microbiology and Biotechnology. 30(5). pp. 279-291.
14. Samanta A.K., Jayapal N., Kolte A.P., Senani S., Sridhar M., Dhali A., Suresh K.P., Jayaram C. and Prasad C.S. (2015). Process for enzymatic production of xylooligosaccharides from the xylan of corn cobs. Journal of Food Processing and Preservation. 39(6). pp. 729-736.
15. Samanta A.K., Jayapal N., Kolte A.P., Senani S., Sridhar M., Suresh K.P. and Sampath K.T.(2012). Enzymatic production of xylooligosaccharides from alkali solubilized xylan of natural grass (Sehima nervosum). Bioresource technology. 112. pp.199-205.
16. Sanz ML, Polemis N, Morales V, Corzo N, Drakoularakou A, Gibson GR & Rastall RA (2005). In vitro investigation into the potential prebiotic activity of honey oligosaccharides. Journal of Agricultural and Food Chemistry. 53(8). pp. 2914-2921.
17. Singh DP and Prabha R (2017). Bioconversion of Agricultural Wastes into High Value Biocompost: A Route to Livelihood Generation for Farmers. Advances in Recycling & Waste Management. 2(3).
18. Vazquez MJ, Alonso JL, Dominguez H & Parajo JC. (2000). Xylooligosaccharides: manufacture and applications. Trends in Food Science and Technology. 11(11). pp.387-393.
19. Yang CH, Yang SF AND Liu WH (2007). Production of Xylooligosaccharides from Xylans by Extracellular Xylanases from Thermobifida fusca. Journal of Agricultural and Food Chemistry. 55(10). pp. 3955-3959.
20. Yuan QP, Zhang H, Qian ZM & Yang XJ (2004). Pilot-plant production of xylooligosaccharides from corncob by steaming, enzymatic hydrolysis and nanofiltration. Journal of Chemical Technology and Biotechnology. 79(10). pp. 1073-1079.