Extraction of Xylooligosaccharides (XOS) from agricultural waste, determining its prebiotic properties and organoleptic qualities of Indian traditional foods upon its addition

Synopsis of PhD Thesis

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Introduction

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 potentially be used to produce various value added products like bio-fuels; animal feeds etc. and can be utilized to manufacture several such products including Xylooligosaccharide (XOS) which may exhibit prebiotic effect when consumed regularly. There is emerging evidence that functional foods ingredients can have an impact on a number of gut related diseases and dysfunctions (A.A. Aacharya and S.G. Prapulla, 2011).

A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers health benefits (Gibson et al, 2004). Besides providing the health benefits, prebiotics are known to extend technological advantages in favour of improved organoleptic qualities of the food products. Xylooligosaccharides (XOS) is a stable prebiotic which can withstand heat up to 100°C under acidic conditions (pH=2.5-8) and has a potential to be incorporated into food products (Courtin et al, 2009).

However, its prebiotic properties needs to be established in terms of bile resistance, acid tolerance, fermentability to produce short chain fatty acids (SCFA) and growth of *Lactobacillus plantarum* and *Bifidobacterium adolescentis and Escherichia coli*. XOS also needs to be exploited for its potential to be incorporated into various food products and study their organoleptic properties similar to fructooligosaccharides (FOS) which have proven technological benefits in terms of its miscibility and organoleptic qualities.

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.

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).

XOS also needs to be exploited for its potential to be incorporated into various food products and study their organoleptic properties similar to fructooligosaccharides (FOS) which have proven technological benefits in terms of its miscibility and organoleptic qualities.

However, after reviewing the literature, it was found that there are limited researches being conducted on XOS and only one research paper by S. Mumtaz et al, 2008 was found on food product development. This also gives a future scope to study the organoleptic properties of XOS upon its addition in different food products.

Rationale

XOS is known to be a potential prebiotic with several health benefits; however we need to find sources for its high content. Most agricultural wastes which have no economic value can be converted to more valuable products such as XOS which may benefit the fruit, vegetable and oil industries to exploit the use of their waste products and convert it into value added products and thereby add to the country's economic growth. Besides providing the health benefits, oligosaccharides are known to extend technological advantages in favour of improved organoleptic qualities of the food products. XOS needs to be exploited for this purpose as it has its chemical and structural qualities much similar to fructooligosaccharides (FOS) which have proven technological benefits in terms of its miscibility and organoleptic qualities.

However, after reviewing the literature, it was found that there are limited researches being conducted on XOS and only one research paper by S. Mumtaz et al, 2008 was found on food product development. This also gives a future scope to study the organoleptic properties of XOS upon its addition in different food products.

Objectives

Broad objective:

"To extract Xylooligosaccharides (XOS) from agricultural waste, determine its prebiotic properties and organoleptic qualities of Indian traditional foods upon its addition."

Specific objectives:

1. To extract Xylooligosaccharides (XOS) from agricultural wastes such as green banana peels, green pea shells, corn cobs and orange peels.
2. To determine the XOS yield by each of the agricultural wastes selected.
3. To determine the prebiotic properties of XOS in vitro.
4. To study the organoleptic properties of XOS upon its addition on a few Indian traditional foods such as Prawn patia, Paneer butter masala, Black rice kheer and Gajar ka halwa.

Review of Literature

This chapter will focus on the available literature under following heads-

- Agricultural produce in India- statistics.
- Agro waste from different edible plants.
- Importance of prebiotics in health.
- Mechanism of its action.
- Types of prebiotics.
- XOS as a prebiotic.
- Methods of XOS extraction.
- Sensory evaluation of food products
    - Types of tests.
    - Selection of Panel Members.
- Technological benefits of adding prebiotics in foods.
- Prebiotic potential of XOS.
- Future scope of prebiotics
    - Food industries

Methodology

This research study was divided into 3 phases.

PHASE I: Extraction of xylooligosaccharide from selected agricultural wastes.

PHASE II: Determining the prebiotic properties of XOS in terms of bile resistance, acid tolerance, growth of *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli*; production of short chain fatty acids (SCFA) such as acetate, butyrate and propionate.

PHASE III: Organoleptic evaluation of XOS added *Prawn Patia*, *Paneer Butter masala, Black Rice Kheer* and *Gajar Ka Halwa* with 5g, 8g and 10g XOS.

PHASE I: Extraction of xylooligosaccharide from selected agricultural wastes.

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

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 Corncob (SUGAR 75). All the samples were procured in April, 2017.

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^{o}C-70^{o}C for 12h in a hot air oven. The pea shells were dried at 65^{o}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.

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 following formula shown in fig. 1

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

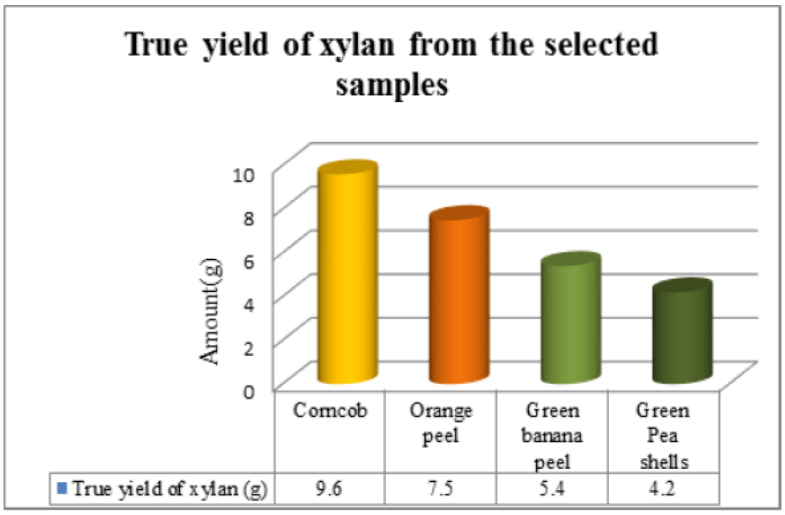


Fig. 1: True yield of xylan from corncob, orange peel, green banana peel and green pea shells.

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

Corncob (S1) = 9.60/4 = 2.40 g, Orange peel (S2) = 7.50/4 = 1.87 g,

Green banana peel (S3) = 5.40/4 = 1.35 g, Pea shell (S4) = 4.20/4 =1.05 g.

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^{o}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.

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^{o}C on Rota evaporator under vacuum. The pure XOS contains xylose, xylobiose, xylotriose, xylotetrose , xylopentose etc. The XOS obtained was dried at 50^{o}C using air tray dryer.

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:

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

PHASE II: Determining the prebiotic properties of XOS in terms of bile resistance, acid tolerance, growth of *Lactobacillus plantarum*, *Bifidobacterium adolescentis* and *Escherichia coli*; production of short chain fatty acids (SCFA) such as acetate, butyrate and propionate.

Materials for bile resistance and acid tolerance test of XOS

Commercial XOS derived from corn cobs were purchased from Hangzhou Focus Corporation. (Hangzhou, China) and was 95% pure, Ox bile and hydrochloric acid procured from Sigma.

Bile resistance test of XOS

Ox bile (1g) was dissolved in 100ml DI water and stirred well till it dissolved. Bile solution was made up to bile level 0.5%, 1% and 1.5% using Ox bile (Sigma) and 5g XOS was added to the bile solutions at room temperature. These solutions were used to study bile resistance

of XOS at 0h, 1.5h and 3h. The samples were filtered and 20 µl each were used for the HPLC analysis. This method was modified in house with reference to (R. M. Duar, 2011).

Acid tolerance test of XOS

XOS (5g) were dissolved in 100ml DI water and stirred well till it dissolved. X ml (QS) Hydrochloric acid solution was added to the solution to adjust pH = 1.5, 2.0 and 3.0. These solutions were used to study acid tolerance of XOS at 0h, 1.5h and 3h. The samples were filtered and 20 µl each were used for the HPLC analysis. This method was modified in house with reference to (R. M. Duar, 2011).

Materials for determination of prebiotic effect of XOS on *L. Plantarum, B. Adolescentis, E. Coli* and SCFA analyses using HPLC

Commercial XOS derived from corn cobs were purchased from Hangzhou Focus Corporation. (Hangzhou, China) and was 95% pure. The degrees of polymerization of the XOS mixture ranged from xylobiose to xylohexaose. All chemicals were purchased from Sigma-Aldrich, India. Bacterial culture for *Lactobacillus Plantarum* strain was purchased from MTCC repository, *Bifidobacterium Adolescentis* strain from National collection of Dairy culture, National Institute of Dairy Research, Karnal. *Escherichia coli* were isolated from sewage at Institute of Science, Nirma University.

Bacterial strains

Lactobacillus plantarum strain (MTT2621), *Bifidobacterium adolescentis* strain (NCDC236) were used in the present study. The bacterial pathogen used was *Escherichia coli*.

Prebiotic effect of XOS on *L. Plantarum, B. Adolescentis* and *E. Coli*

Lactobacillus plantarum was grown in MRS broth in anaerobic jar, *Bifidobacterium adolescentis* in MRS broth along with 0.05% cysteine in anaerobic jar and *Escherichia coli* in Luria–Bertani (LB) broth at 37°C for 24 h. After 24 h, each bacterium was allowed to grow with XOS concentration 0.5%, 1%, 2%, 3% and 4%. The bacteria were grown on their respective media such as MRS agar and Luria broth without XOS as negative control. 10%v/v inoculation was added from the active culture having OD between 0.08 and 0.1 at 620nm.

These were then incubated at 37°C for 24 h. After 24 h, readings were taken in Spectrophotometer (Agilent, model no: carry 60) at 620nm.Prior to each OD measurement the flasks were carefully shaken. The concentration of XOS which gave maximum OD for each bacterium was further chosen for SCFA analysis using HPLC. All measurements were performed in duplicates.

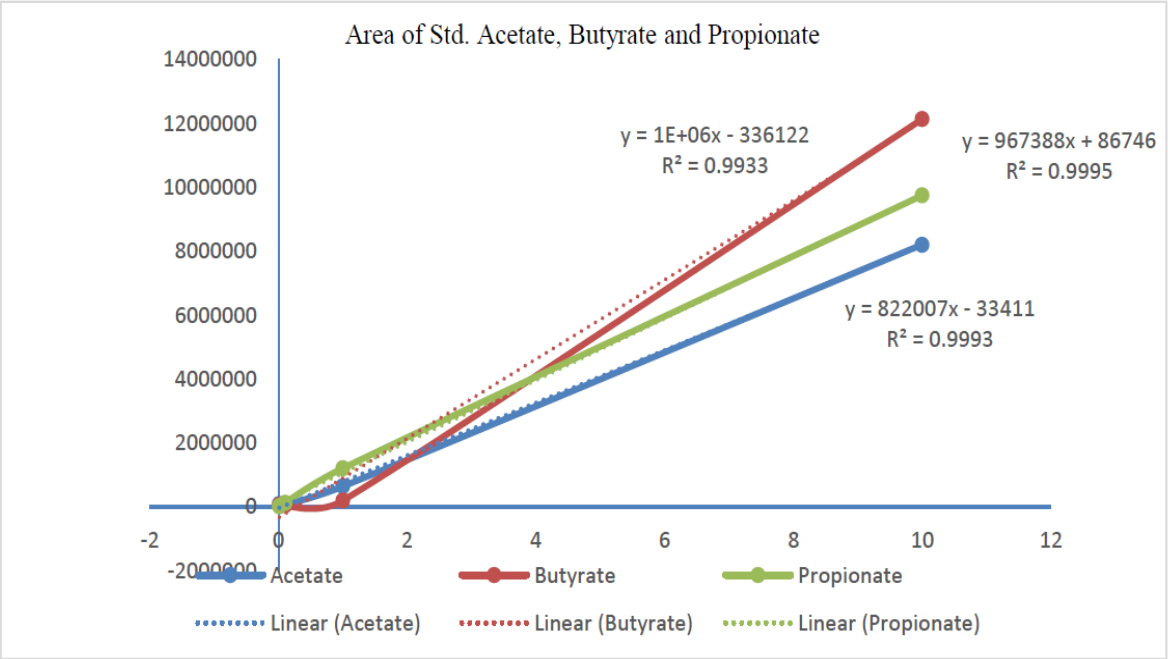
SCFA analyses using HPLC

To evaluate the efficiency of the fermentation of XOS by *Lactobacillus plantarum* strain (MTT2621), *Bifidobacterium adolescentis* strain (NCDC236) and *E. coli*, HPLC was performed. Acetic, Butyric and propionic acids, products of the XOS fermentation, can be detected in the growth medium and quantified by HPLC.

The first step of the experimental set-up was choosing the appropriate column for efficient separation of the analytes. The column Phenyl hexyl, 100 × 4.6 mm (Agilent technologies, USA) was chosen as it was prepared for separation of small polar compounds such as short-chain fatty acids. This HPLC consists of UV210 detector (Shimadzu, Kyoto, Japan) connected to a recorder. Separation of the analytes took place in the aforementioned Phenyl hexylcolumn. The peaks for analysis were obtained in the computer software connected to it.

24 hour old culture was centrifuged at 7,000 rpm for 5 mins. Supernatant was diluted (1:1) in buffer containing 2.5pH water using H3PO4. 10mg/ml of standards of acetate, butyrate and propionate (HPLC grade, Sigma) were run at 1mg/ml flow rate in the HPLC till 15 minutes and 20µl of samples/standard were injected in phenyl hexyl column (Agilent). Detection wavelength was 210 nm and recording range was set to 0.2 absorbance unit's full scale.

Prior to use, the mobile phase and samples were filtered through 0.2un filter prior to injecting the samples in the column. Fig shows the area of standard Acetate, Butyrate and Propionate. Area was calculated for the standards and SCFA samples were calculated based on the area of standards.



PHASE III: Organoleptic evaluation of XOS added *Prawn Patia*, *Paneer Butter masala*, *Black Rice Kheer* and *Gajar Ka Halwa* with 5g, 8g and 10g XOS.

Procurement of raw materials

For *Black rice kheer*:

Black rice was procured directly from a farmer from Sonitpur district, Assam, India. Amul Taaza milk, sugar, cashews, almonds, raisins were procured from a supermarket in Guwahati, Assam.

For *Prawn patia*:

Deveined medium sized prawns, mustard oil, curry leaves, onion, small green chillies, tomato puree, ginger, garlic, red chilli powder, turmeric powder, cumin powder, granulated sugar, vinegar and salt were procured were procured from a supermarket in Guwahati, Assam.

Commercial XOS derived from corn cobs were purchased from Hangzhou Focus Corporation. (Hangzhou, China).

For *Gajar Ka Halwa*:

Sugar, milk, cardamom, ghee and Khoya were procured from a supermarket in Guwahati, Assam. Carrot was purchased from a local market in Guwahati, Assam.

For *Paneer Butter Masala*:

Paneer, onions, ginger, garlic, tomatoes, cashew nuts, bay leaf, green chillies, full fat milk, garam masala powder, coriander powder, red chili powder, sugar, fresh cream, butter, oil and salt were procured from a supermarket in Guwahati, Assam.

Preparation of *black rice kheer*

Ingredients:

Black rice – 30g

Milk – 250ml

Sugar – 30g

Almonds chopped – ½ tbsp

Cashew nuts chopped– ½ tbsp

Raisins - 1 tsp

Preparation:

1. Black rice was washed under running water till water runs clear. Rice was soaked in enough water overnight. Nuts were chopped and kept aside.
2. Milk was taken in a heavy bottom pan and Turn the heat on medium. Let the milk come to a boil.
3. Rice was added once the milk started boiling. It was allowed to simmer on low-medium heat 25-30 minutes with continuous stirring.
4. Thirty grams of sugar and chopped cashews, almonds and raisins was added to it and mixed well. It was allowed to simmer for 8-10 minutes. The stove was turned off when the Kheer was ready.
5. Sample A was the standard recipe with 0g XOS and 30g sugar. Sample B had 5g XOS and 25g sugar, Sample C had 8g XOS and 22g sugar and Sample D had 10g XOS and 20g sugar.

Preparation of *prawn patia*

Ingredients:

Vegetable oil- 2 tbsps

Finely chopped medium sized onion- 1 no.

Chopped green chillies- 4 nos.

Tomato puree- 100 g

Ginger-garlic paste- 1 tbsp

Red chilli powder- 1 ½ tsp

Turmeric powder- 1 tsp

Cumin powder- 1 tsp

Granulated sugar- 30 g

Vinegar- 1 ½ tbsp

Salt- as preferred

Medium sized deveined frozen prawns (Sumeru) - 1 cup

Preparation:

1. Frozen prawns were thawed. Onions, chillies were peeled, washed and chopped.
2. Mustard oil was heated oil in a skillet over medium-high heat until simmering. Chopped onions were added and stirred until it turned light brown. Green chillies were added and stirred for a minute.
3. Tomato puree was added and continued stirring until oil separated from the onion tomato mix. Ginger-garlic paste, red chilli, turmeric and cumin were added and stirred ensuring that the mixture doesn't stick to the bottom.
4. Sugar, vinegar and water was added and cooked till the rawness of the tomato is gone. It was seasoned with salt to taste. Prawns were added, stirred and cooked for 10 more minutes until the prawns were cooked.
5. The stove was turned off when it was ready.
6. Sample A was the standard recipe with 0g XOS and 30g sugar. Sample B had 5g XOS and 25g sugar, Sample C had 8g XOS and 22g sugar and Sample D had 10g XOS and 20g sugar.

Preparation of *Paneer Butter Masala*

Ingredients:

Paneer - 100 gms

Onions, finely chopped- 2 medium

Ginger, chopped - 1 tsp

Garlic- 3-4 Cloves

Tomatoes- 2 medium

Cashew Nuts, soaked in water for 15 minutes- 6-8

Bay Leaf- 1 small piece

Green chillies - 2 nos.

Full Fat Milk- $1/4^{th}$ cup

Garam Masala Powder- 1/2 tsp

Coriander Powder- 1 tsp

Red Chili Powder- 1/2 tsp

Sugar- 30g

Fresh Cream- 1 tbsp

Butter- 1 tbsp

Oil- 1 tbsp

Salt to taste

Preparation:

1. Frozen paneer was thawed.
2. Onion, ginger and garlic were grounded in a grinder and made into a paste. Soaked cashew nuts were grounded into a paste with 2 tbsps of water to make a smooth paste.
3. Tomatoes were blanched and blended into a tomato puree.
4. Cooking oil and butter was heated in a non-stick frying pan over medium flame.
5. Onion paste and bay leaf was sautéd until onion paste turned light brown for approx. 4-5 minutes.
5. Green chillies and red chili powder was added and sautéd for 30-40 seconds. Cashew nut paste was added and sautéd and cooked for 2 minutes.
6. Tomato puree and sugar was added and cooked until oil started to separate from the puree, it took around 5 minutes.
7. Coriander powder and garam masala powder was added and mixed well.
8. 1/2 cup milk, 1/2 cup water and salt was added; mixed and cooked until oil came on the surface.
9. Paneer cubes were added and cooked for approx 3-4 minutes or until the desired consistency of gravy.
10. Fresh cream was added and mixed properly and the flame was turned off.
12. Paneer butter masala is ready garnished with milk cream or cube of butter and serve.
13. Sample A was the standard recipe with 0g XOS and 30g sugar. Sample B had 5g XOS and 25g sugar, Sample C had 8g XOS and 22g sugar and Sample D had 10g XOS and 20g sugar.

Preparation of *Gajar Ka Halwa*

Ingredients:

Ghee, melted- ½ cup

Carrot, grated - 1 kg

Evaporated milk- 1 cup

Sugar- 30g

Khoya- 50g

Almonds, soaked in hot water, peeled and slivered- 15

Cardamom powder- ½ tsp

Preparation:

1. Grated carrots and milk were boiled in a large heavy based pan. Carrots were cooked and stirred constantly, until all the milk dried up.
2. Ghee was added to the carrots. Khoya was added to it and sautéd for a few minutes.
3. Sugar was added to it and continued to cook and stirred till it dried.
4. It was removed from heat when still moist and stirred in the cardamom powder and almonds reserving a few to garnish.
5. Sample A was the standard recipe with 0g XOS and 30g sugar. Sample B had 5g XOS and 25g sugar, Sample C had 8g XOS and 22g sugar and Sample D had 10g XOS and 20g sugar.

Sensory evaluation

Thirty panelists were screened in three successive trials through threshold test at The Institute of Hotel Management, Catering Technology and Applied Nutrition, Guwahati, Assam. Sensory evaluation was carried out on *Black rice kheer*, *Gajar Ka Halwa*, *Prawn patia and Paneer Butter Masala* samples containing 0g, 5g, 8g and 10g of XOS. Out of thirty screened panelists, 25 semi trained panelists were selected using the sensitivity threshold test. The panel members were asked to fill the questionnaire and rate the samples for Colour and appearance, texture, taste and mouthfeel, aftertaste and overall acceptability using a composite score analysis in triplicates.

Results and discussion

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^{o}C for 5h.

In the present study, crude xylan yield 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^{o}C) or autoclaving (121^{o}C, 15 lbs, 45min).

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.

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 and 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^{o}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. The present study revealed that pure XOS obtained from 100g dry powdered samples of corncob, orange peels, raw green banana, and green pea shells were 3g (18.75%), 2.35 (26.11%), 1.68g (13.46%), and 1.31g (18.80%) respectively. Although all the four samples yielded high amounts of XOS, orange peels yielded the highest.

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^{o}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.

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.

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).

Bile resistance test of XOS

In the present study, no degradation of XOS was observed on exposure of XOS to bile at 0h, 1.5h and 3h with bile concentration 0.5%, 1% and 1.5%. The tests were carried out in duplicates.

A study on pH stability of prebiotic non-digestible wheat bran-derived arabinoxylooligosaccharides (AXOS), xylooligosaccharides (XOS)-and chicory root inulin-derived fructooligosaccharides (FOS) were compared. Decomposition was revealed at alkaline pH (pH 11.0) for all three preparations tested. The short chain oligosaccharides, XOS and FOS were more sensitive to alkaline decomposition than were the longer chain AXOS, the latter being the result of the higher abundance of reducing ends in short chain oligosaccharide preparations (Courtin M.C et al, 2009).

Acid tolerance test of XOS

In this study, XOS recovery was observed to be 100% on its exposure to pH 1.5, 2 and 3 at 0h. At 1.5h recovery of XOS was found to be 98.4%, 98.9% and 97.9% at 1.5pH, 2pH and 3pH respectively. XOS recovery was 96.2%, 97.3% and 96.3% on its exposure to 1.5pH, 2pH and 3pH respectively at 3h. The tests were carried out in duplicates.

At pH 2.0 and 3.0, hydrolysis of oligosaccharide linkages took place, with FOS being the most acid-sensitive component (Courtin MC et al, 2009). Recoveries were 100%, 91% and 113% for the supplemented muffin, cookie and nutrition bar, respectively at 3.5 pH. For the breakfast cereal, only 47% of the supplemented FOS remained after extrusion at optimal conditions (170 rpm and 140 °C) (Duar RM, 2011). Whereas, recoveries of Inulin at pH 3.5 were 106%, 103% and 107% and 126% obtained from the supplemented extruded cereal, nutrition bar, sports drink and muffins, respectively (Duar RM, 2011).

Another study on evaluation of the prebiotic effects of citrus pectin hydrolysate (PEH), it was found that when pH was reduced to 3.2, populations of the tested probiotics did not decrease significantly ($p > 0.05$) for all treatments. The tested probiotics showed significantly higher acid tolerance and survival populations in the media supplemented with PEH than glucose. This indicated that PEH should contain some oligosaccharides which assisted the probiotics in acid tolerance and survival ability, while glucose did not (Yen YH et al, 2017).

Cummings JH et al, 2001 reviewed on the digestibility of Inulin and Oligofructose and found an average recovery of 88% in human upper intestine. There is little available information in the literature on bile resistance, acid tolerance properties of XOS *in vitro.*

Prebiotic effect of XOS on the growth of *L. Plantarum, B. Adolescentis* and *E. Coli*

In this study, the growth of *Lactobacillus plantarum (LP)* and *Bifidobacterium adolescentis (BA)* were higher at 0.5%, 1% and 2% of XOS addition. For *Escherichia coli (E.coli)* the growth gradually decreased as the concentration of XOS increased from 0.5% to 2%. Since 0.5%, 1% and 2% levels of XOS concentration gave better or almost equivalent growth of *Lactobacillus plantarum (LP), Bifidobacterium adolescentis(BA)* and reduced the growth of *Escherichia coli (E. coli).* Therefore, 0.5%, 1% and 2% levels of XOS concentration samples were chosen for production of short chain fatty acids (SCFA) and its analysis.

Mean growth of *Lactobacillus plantarum* was more with 0.5% and 1% XOS concentration at $p \leq 0.01$, growth of *Bifidobacterium adolescentis* was seen to be same with 0.5% and 2% XOS concentration at $p \leq 0.01$ and growth of *Escherichia coli* was least with 1% XOS.

A study on functional properties of commercial prebiotics showed the increase in cell density of *L. paracasei 1195* grown on Raftilose P95, Inulin-S, and Raftiline HP were significantly higher ($p \leq 0.05$) than for glucose. *B. bifidum NCI* had a significantly higher ($p \leq 0.05$) increase in cell density when grown on NutraFlora P-95 and Raftilose P95 than on glucose. Also, the increase in cell densities of *L. plantarum 4008* and *L. acidophilus 33200* were significantly larger ($p \leq 0.05$) for purified GOS than for glucose (Huebner J. Et al, 2007).

An in vitro study investigated the potential prebiotic effect of natural (NS) and blanched (BS) almond skins, the latter being a by-product of the almond-processing industry. Their study concluded that dietary fibre from almond skins altered the composition of gut

bacteria and almond skins resulting from industrial blanching could be used as potential prebiotics (Mandalari G. et al, 2009).

A study on the prebiotic activity of XOS obtained from corncob and reagent grade xylan were tested in *L. brevis, L. plantarum, L. acidophilus, L. rhamnosus* cultures, and in a co-culture with *Escherichia coli* as a challenge microorganism to prove the bacteriostatic activity of lactobacilli strains. Xylooligosaccharides stimulated *L. brevis* and *L. plantarum* growth: these microorganisms grew faster than the other lactobacilli strains. *L. acidophilus* grew better in the presence of XOS and maintained the absorbance of the culture. In the co-culture in presence of both XOS the challenge microorganism did not grow; lactobacilli colonies appeared in MRS agar. No colonies of *E. coli* grew in EMB plaques (Pedraza L. et al, 2014).

SCFA production analysis during fermentation in vitro

Lactobacilli and Bifidobacteria ferment carbohydrates through a pathway mediated by the glycolytic enzymes in which the main end products are SCFA (Grootaert et al., 2007). Butyrate, Propionate and Acetate are the major SCFA produced during fermentation of carbohydrates in the large bowel (Maniserri C et al, 2009).

A study on bioactive xylooligosaccharides from wheat bran soluble polysaccharides reported that Acetate was the chief SCFA liberated due to in vitro fermentation of xylooligosaccharides (Maniserri C et al, 2009).

Another study on prebiotic effects of Xylooligosaccharides on the improvement of microbiota balance in human subjects reported that the abundance of pathogenic bacteria, *Clostridium perfringens*, was significantly lower in the fecal samples of the XOS group than in those of the control group. This was explained by the XOS suppressing the growth of *Clostridium perfringens*; the mechanisms underlying this effect were likely due to the production of short-chain fatty acids (SCFAs) via the fermentation of XOS in the colon. A decrease in intestinal pH has been reported as a consequence of the increased SCFA production which subsequently inhibits the overgrowth of pathogenic bacteria (Lin SH et al, 2016).

A comparative study of synbiotic and prebiotic supplementation on gut health, SCFA, hs-CRP and lipid profile of type 2 diabetic subjects with pre hypertension concluded that daily intake of 1 g synbiotic product and 10 ml FOS improved gut health, hs-CRP, lipid

profile and short chain fatty acids (SCFA) of the subjects which may be due to increased production of SCFA (Sheth M et al, 2016).

Another study on consumption of XOS in combination with inulin did not decrease the concentrations of acetate and *p*-cresol, but increased the faecal concentrations of total SCFA and propionate (Lecerf JM et al, 2012).

In this study, Acetate was produced the most followed by Propionate and Butyrate. *Bifidobacterium adolescentis* produced acetate (1833mMol), butyrate (343.28mMol) and propionate (408.67mMol).

Lactobacillus plantarum produced acetate (1883.82 mMol), butyrate (340.72mMol) and propionate (405.62mMol).

Escherichia coli produced acetate (324 mMol), butyrate (339.55 mMol) and propionate (285.22 mMol).

Bifidobacterium adolescentis produced (331%) more of Butyrate and Propionate respectively on its exposure to XOS ($p \leq 0.01$), whereas, *Lactobacillus plantarum* produced more acetate as compared to *Bifidobacterium adolescentis* ($p \leq 0.001$). Production of all the three SCFA reduced (20%-48%) in case of *Escherichia coli* on its exposure to XOS ($p \leq 0.001$).

Organoleptic evaluation of *Black rice kheer, Gajar Ka Halwa, Paneer Butter Masala* and *Prawn patia*

F test revealed no significant difference the organoleptic scores of XOS added *Black rice kheer, Gajar Ka Halwa, Paneer Butter Masala* and *Prawn patia* at all levels of addition (5g, 8g and 10g) prepared by substituting sugar with varying levels of XOS. Hence, XOS addition to these products was well accepted by the panelists up to 10g level of addition.

A study conducted on development and sensory analysis of a buttermilk based fermented beverage using barley and fructooligosaccharide as functional ingredients reported high scores for overall acceptability and the sweet taste of FOS did not negatively affect the taste, aftertaste and mouthfeel of the product (Sheth M et al, 2016). Another study conducted on FOS added beverages and soup namely, butter milk, lemon juice, milk and tomato soup at 2.5%, 4%, 5%, 6%, 7.5% showed positive results on the overall acceptability of the products (Neha G et al, 2011). Similar results were reported by Parnami *et al*, where cookies and bread were fortified with prebiotic inulin (Parnami S et al, 2010).

A study on Xylooligosaccharide enriched yoghurt reported that addition of XOS up to 3.5% did not influence taste and overall acceptability but higher levels of addition resulted in lower after taste scores (Mumtaz S et al, 2008).

However, difference test conducted to determine if the products judged were superior, equal or inferior to the standard product (0% XOS) with varying levels of XOS revealed that most of the panelists found color of the *Black rice kheer* to be superior or equal to the standard ($p \leq 0.01$) at all the three levels of addition. The overall acceptability and other sensory attributes of Black rice kheer were equal or superior at 8g and addition of 10g XOS rendered *Black rice kheer* less sweet. This data indicated that XOS improved most of the organoleptic attributes up to 8g of addition.

In *Prawn patia,* difference test revealed that the sensory attributes of *Prawn patia* with different levels of addition of XOS were either superior or equal to the standard product ($p \leq 0.001$).

In *Paneer Butter Masala*, most of the panelists found its taste to be superior or equal to the standard ($p \leq 0.001$) at all the three levels of addition. The overall acceptability and other sensory attributes of *Paneer Butter Masala* were equal or superior at 8g.

Most of the panelists found the taste of *Gajar Ka Halwa* to be superior or equal to the standard ($p \leq 0.01$) at all the three levels of addition. The overall acceptability and other sensory attributes of *Gajar Ka Halwa* were equal or superior at 8g and addition of 10g of XOS made it equally acceptable as compared to the standard.

Conclusion and Recommendations

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 that benefit human health. Few researchers have explored edible plants such as corncobs, sugarcane baggase, rice husk and wheat husk to extract xylooligosaccharide.

The present study for the first time has tried to explore green pea shells and green banana peels to check the presence of XOS. Few studies on corncob and other products have reported different levels of XOS content and there might be a correlation between varietal differences and XOS content. Hence, there is a great scope to explore several varieties of agricultural products.

Alkaline extraction method needs to be explored further and to optimize the yield of XOS with different levels of pH, temperature enzyme dosage and different varieties. The method can be further modified for industrial set up and manufacture XOS out of agro waste at commercial level so as to raise country's economic growth by making it available at national and global markets.

The prebiotic potential of XOS in terms of acid tolerance, bile resistance, growth of probiotic bacteria and production of SCFA was successfully established in this study. XOS was well accepted by the panel members for all the four products up to 10g of addition indicating that many foods may be enriched with XOS as a prebiotic and studied for their organoleptic acceptability.

However, further in vivo studies shall be undertaken to demonstrate the clinical efficacy of XOS intake with respect to various non communicable diseases.

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