CHAPTER-4
MATERIALS AND METHODS

Recently there has been a significant increase in research on reducing wastes, saving the environment, utilizing agro wastes for value added products benefitting mankind and the environment hand in hand. 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. The present study was conducted with the primary objective to extract Xylooligosaccharides (XOS) from agricultural wastes, determine its prebiotic properties and organoleptic qualities of Indian traditional foods upon its addition.This chapter outlines the experimental design and discusses the methods and materials used to fulfil the objectives of the study under the following heads.

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.

Experimental design

Phase I:

(a) Procurement and primary processing of agricultural waste.

1) Selection of agricultural wastes from the research station:

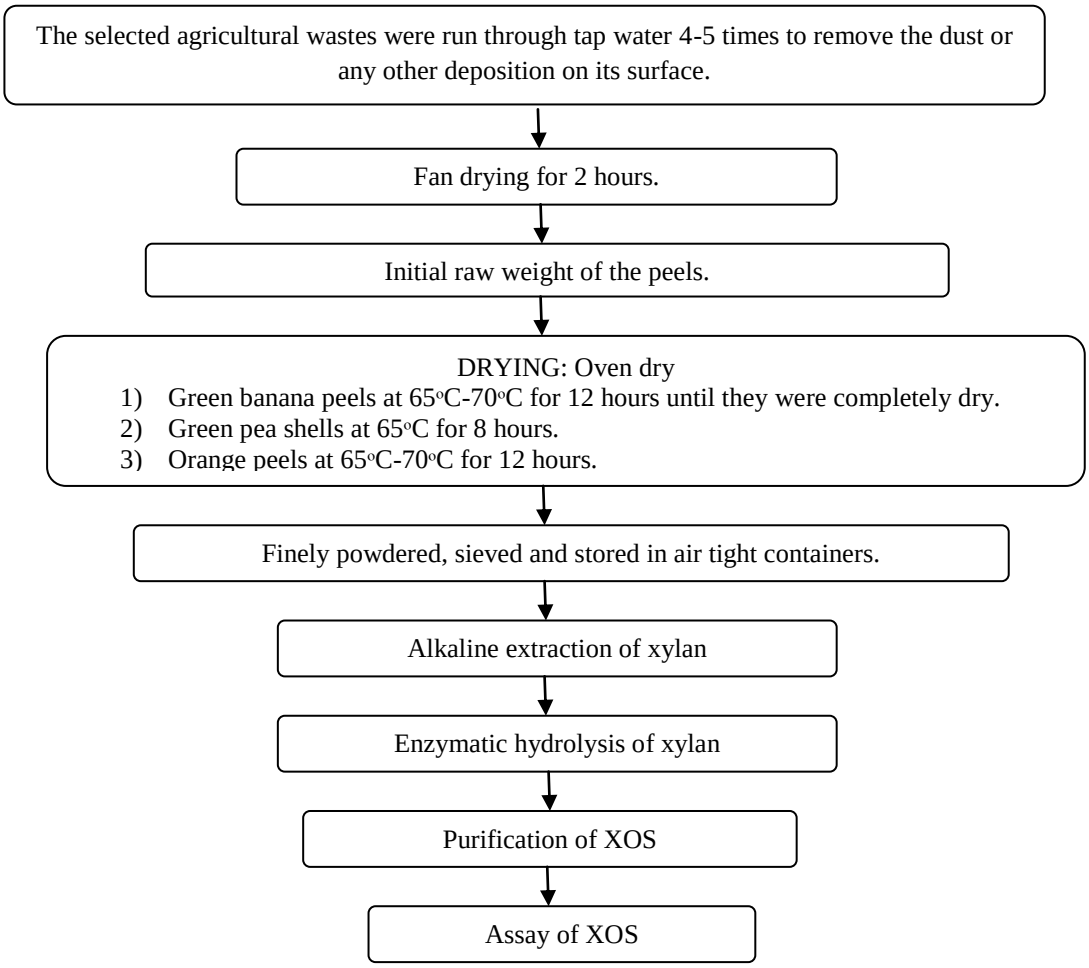
The varieties of the following selected agricultural products used by the food industries of Gujarat for the processing of various processed food products were collected from the research station, Anand Agricultural Model Farm, Vadodara, Gujarat.

a) Green Banana peels, (b) Green pea shells, (c) Orange peels, (d) Corncob powder were procured from Rahi Industries, Mehsana.

2) Identification of the selected variety:

The varietal names were identified by the Agricultural Officer from Anand Agricultural University Model Farm.

(b) **Preparation of the substrate for XOS extraction and analysis.**



Phase: II: Determining the prebiotic properties of XOS in vitro.

(a) Acid test

(b) Bile test

(c) Growth of Probiotic bacteria: *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli*

(d) Production of Short chain fatty acids (SCFA): Acetate, butyrate and propionate.

Phase III: Organoleptic evaluation of XOS added Indian traditional foods

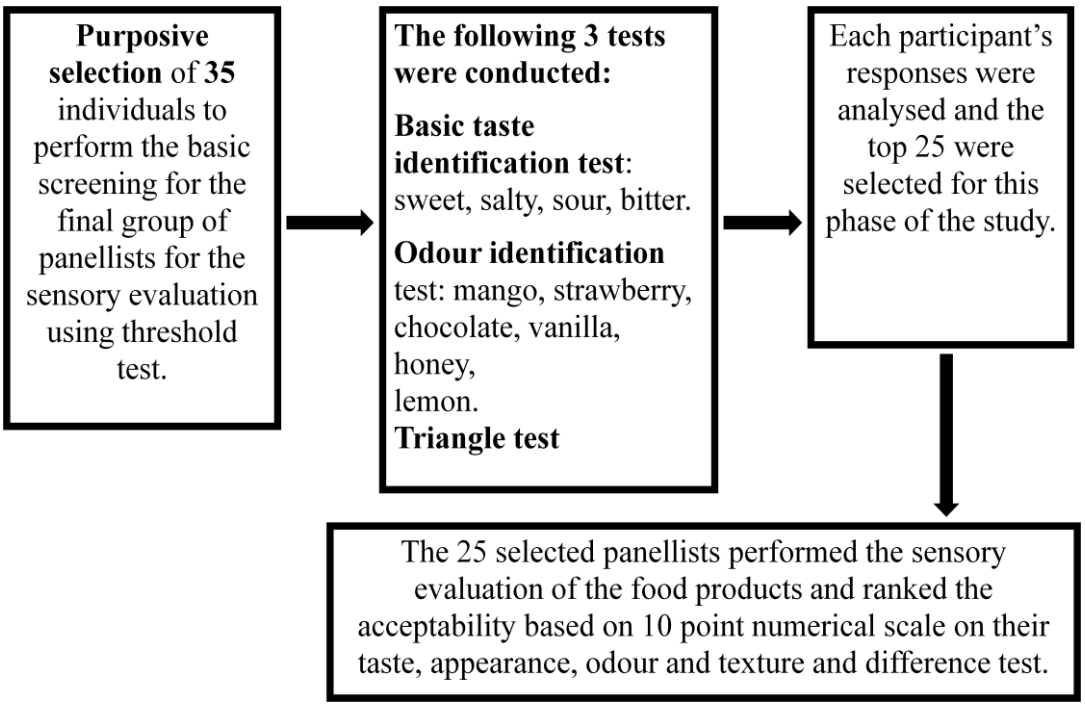
(a) Selection of the food products:

i) Paneer butter masala ii) Prawn patia iii) Black rice kheer iv) Gajar ka halwa

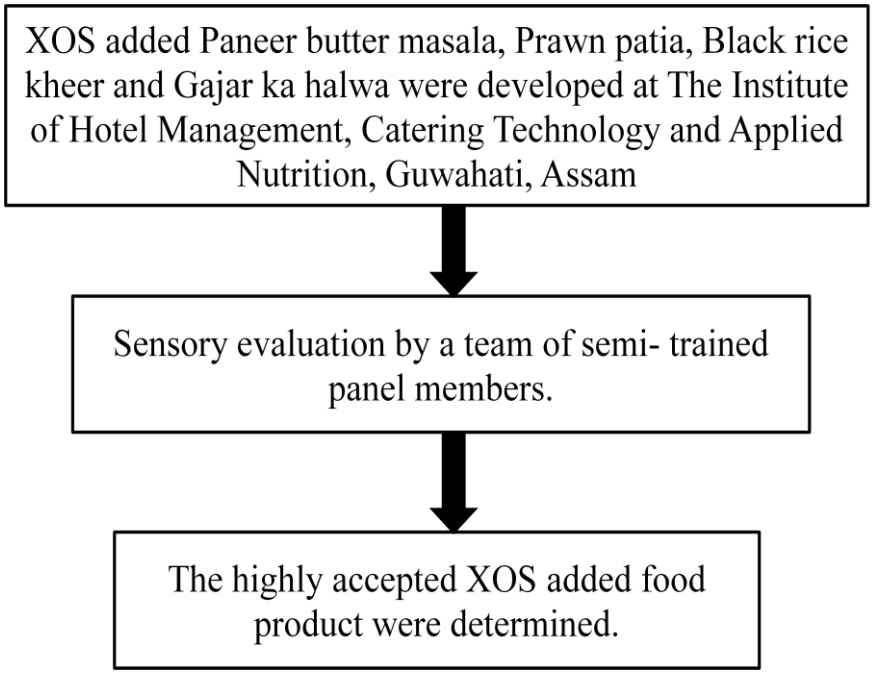
(b) Standardization of the four selected recipes.

(c) Substitution of sugar with XOS into the four food products at 5gms, 8gms and 10gms were done.

(d) Sensory evaluation by semi-trained panel members.



(B. Srilakshmi, 2018)



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.

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

4.1.2. 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^{o}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.

4.1.3. 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 following formula shown in fig. 4.1.

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

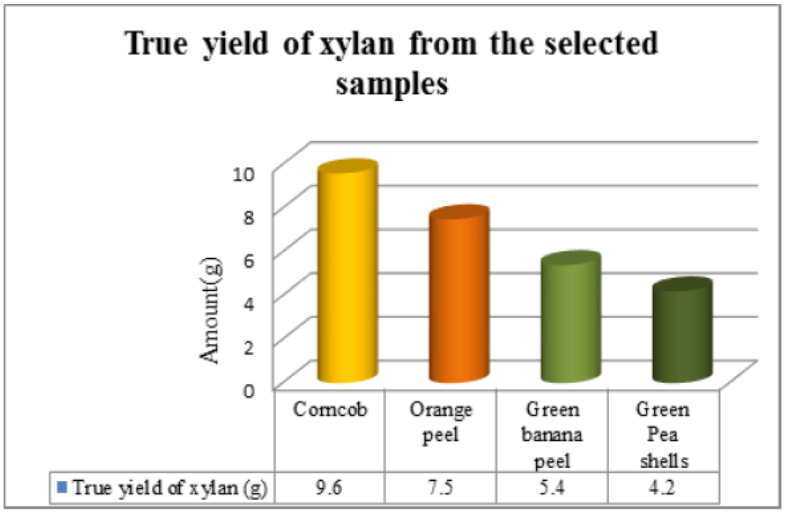


Fig. 4.1. True yield of xylan from corncob, orange peel, green banana peel and green pea shells.

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.

4.1.4. 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 Sigma, 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 (Broekaert et al, 2011).

4.1.5. 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 28g 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.

4.1.6. 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:

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

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.

4.2.1. 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.

4.2.1.1. 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 (Duar, 2011).

4.2.1.2. 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 (Duar, 2011).

4.2.2. 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.

4.2.2.1. Bacterial strains

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

4.2.2.2. 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^{o}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.

4.2.3. 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.

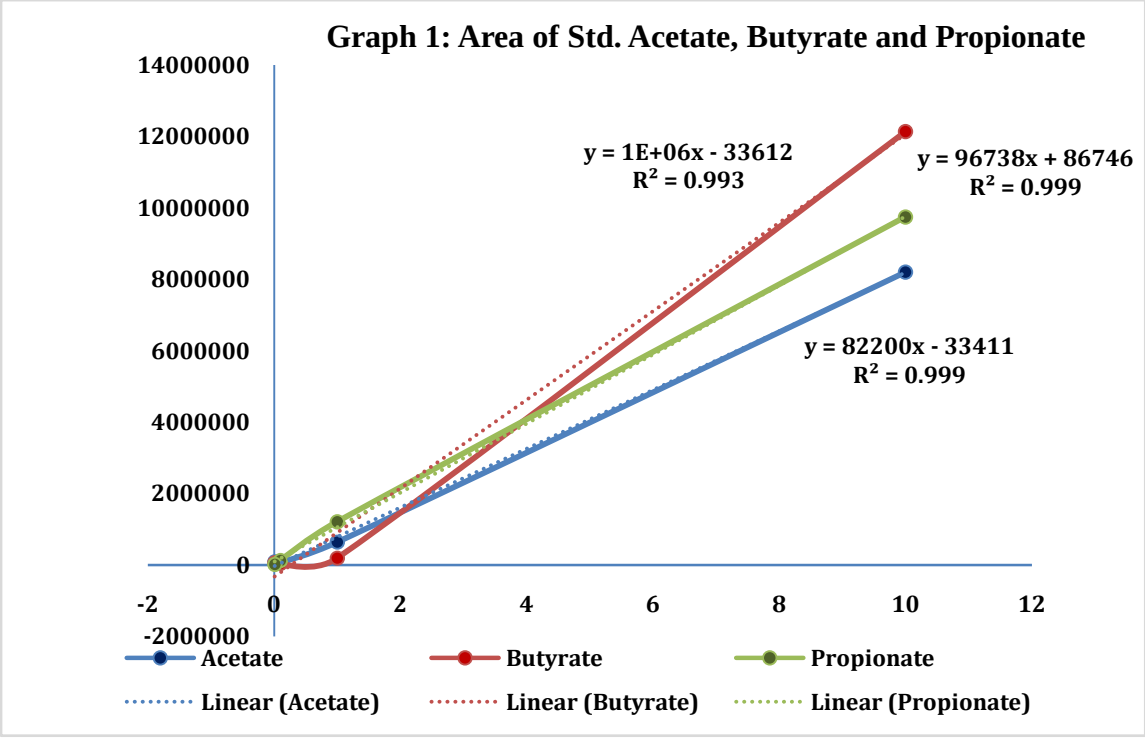
24 hour old culture was centrifuged at 7,000 rpm for 5 mins. Supernatant was diluted (1:1) in buffer containing 2.5pH water using H3PO4. 10mg/ml of standards of acetate, butyrate and propionate (HPLC grade, Sigma) were run at 1mg/ml flow rate in the HPLC till 15 minutes and 20µl of samples/standard were 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. Graph 1, 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.

For example:

				Conversion factor	Dilution factor (Conversion factor × 4)		
			y = 822007x – 33411	*16.9	*df		
	Sample	Area	mg/ml	mMol	mMol	Avg	SD
Acetate	Control1	3094399	3.805089251	64.30600834	257.2240334	223.0231655	48.36733119
	control2	2262643	2.793229255	47.20557441	188.8222976		

Same way for Butyrate and Propionate was done. Conversion factor is to convert mg/ml values to mMol, as research papers express these values in mMol. For butyrate this factor is 11.34 and propionate 13.5. This factor is then multiplied to the y value. Dilution factor is obtained by multiplying conversion factor with 4.



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.

4.3.1. Procurement of raw materials

Food grade XOS derived from corn cobs were purchased from Hangzhou Focus Corporation (Hangzhou, China) in a pack of 6 kgs which was used as a sugar replacer.

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.

Prawn patia is one of those dishes that combine every flavor we normally have separately into one. There are sweet, spicy, tangy and hot notes that work wonderfully with each other.

For *Paneer Butter Masala*:

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

Paneer butter masala also known as Paneer makhani is a slightly sweet creamy dish of paneer, originating from the Indian subcontinent, in which the gravy is prepared usually with butter, tomatoes, cashews or cream. Spices such as red chilli powder and garam masala are also used to prepare the gravy.

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.

Kheer is a rice pudding, originating from the Indian subcontinent, made by boiling milk and sugar with one of the following: rice, broken wheat, tapioca, vermicelli, sweet corn, etc. It is flavored with cardamom, raisins, saffron, cashews, pistachios, almonds or other dry fruits and nuts. It is typically served during a meal or as a dessert.

For *Gajar Ka Halwa*:

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

Gajar ka halwa was first introduced during the Mughal period and the name originates from the Arabic word "halwa", which means "sweet" and it is made from carrot (in Hindi: *gajar*) hence, it is known as gajar ka halwa (meaning pudding of carrot or Halwa of carrot). It is strongly associated with Punjab but it is not clear whether it originated there. Gajar ka halwa originally contained carrots, milk and ghee but nowadays includes many other ingredients like mava (khoya). This age old traditional recipe remained in Punjabi cookbooks for many years.

4.3.2. 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.

Cooked weight: 180g

4.3.3. 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- 1/4th 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.

Cooked weight: 140g

4.3.4. 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.

Cooked weight: 260g

4.3.5. 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.

Cooked weight: 250g

4.4. 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.

4.4.1. Selection and training of panellists for organoleptic evaluation

a) Screening of Panellists: In this section, selection of panellists was carried out. Teaching faculty of The Institute of Hotel Management, Catering Technology and Applied Nutrition, Guwahati, Assam was subjected to threshold testing.

b) Threshold test (Ranganna, 1995)

A stimulus scale at which a transition in a series or judgement occurs is defined as threshold. Score card for the same was formulated and pre tested [Appendix I (i), (ii)]. Two sets of the solution was given to each participants i.e. Set 1 and Set 2 having six solutions of different concentrations of salt and sugar respectively and were arranged in random order. They were asked to identify and rank the samples in increasing order of concentration of taste from the test solutions offered. For screening of the panelists three successive trials were conducted. Participants who cleared the threshold test were included further in evaluating the organoleptic characteristics of the food products.

c) Training of the selected panellists: To impart the basic knowledge and understanding of visual and organoleptic characteristics of the products a training tool was used (ORA Lab Manual, 2013). (Appendix II)

d) Development of score cards for organoleptic evaluation of the food products: Score cards were developed for organoleptic evaluation (Appendix III). Tools for evaluation are mentioned.

4.4.2: Tools for organoleptic evaluation

Organoleptic evaluation tools selected were

a) Numerical Scoring test and
b) Difference test (ISI 1972)

a) Numerical Scoring Test: This test was conducted for visual and organoleptic evaluation. Panelists were asked to evaluate and score essential quality attributes that were needed to be scored like color, taste, after taste etc. The maximum score of each attribute was 10. These tests were conducted to assess each attribute of all the four products for all the samples.

b) Difference Test: To measure the effect of process change on quality of the product or by formulation for product improvement difference test was used. It measures more than one test variable per session. Each panelist was served 4 samples. One sample was known standard. The panelists compared each coded sample with the known standard. The scores that the panelists assigned to the blind standard were subtracted from the score assigned to the test variable. The development and evaluation of XOS incorporated recipes is depicted in plates 4.20 – 4.23. Sample A is the standard recipe. Sample B, C and D has XOS 5g, 8g and 10g respectively.

4.5. Statutory clearances

The Institutional Ethics Committee for Human Research (IECHR) of Faculty of Family and Community Sciences, The M.S. University of Baroda approved the study proposal and allotted the ethical approval number (IECHR/2018/17). Written informed consent was obtained from the participants who agreed to participate in the third phase of the study.

4.6. 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.

The HPLC analysis of XOS recovery on bile resistance and acid tolerance were conducted in duplicates for each of the samples at 0.5%, 1% and 1.5% bile concentration and 1.5pH, 2pH and 3pH respectively. The effect of incubation period on recovery of XOS was conducted at 0h, 1.5h and 3h. Growth of bacteria was conducted in triplicates and OD was measured for each samples. Production of SCFA was also analyzed using HPLC. 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. Chi square was also used to determine if statistical difference ($p<0.05$) existed.

Plate no. 4.1: Green banana peels

Plate no. 4.2: Green banana peels before fan drying

Plate 4.3: Green banana peels in hot air oven for drying

Plate 4.4: Orange peels

Plate 4.5: Dried green pea shells

Plate 4.6: Dried powder of green banana peels

Plate 4.7: Dried powder of green pea shells

Plate 4.8: Dried powder of orange peels

Plate 4.9: Corn cob powder procured from Rahi industries, Mehsana, Gujarat.

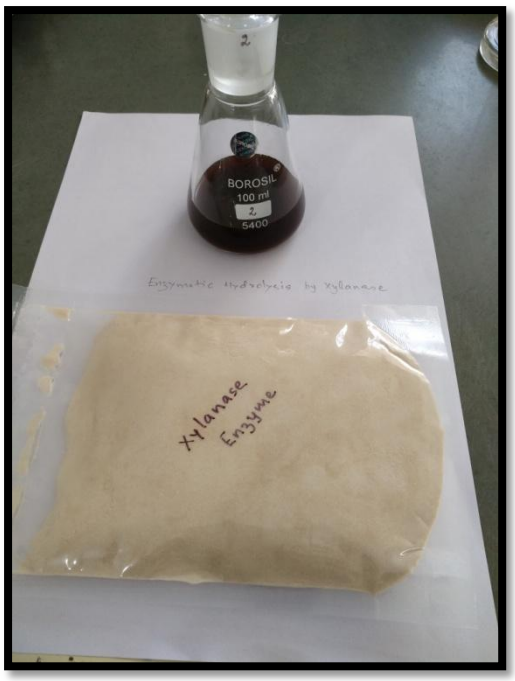


Plate no. 4.10: Xylanase enzyme for enzymatic hydrolysis of xylan

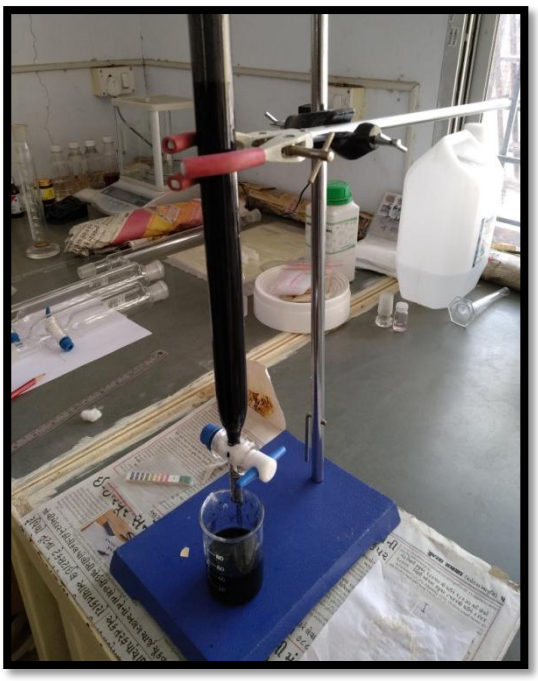


Plate no. 4.11: Twenty eight gms of activated charcoal in a vertical glass column of diameter 26mm and length 450mm for purification of XOS

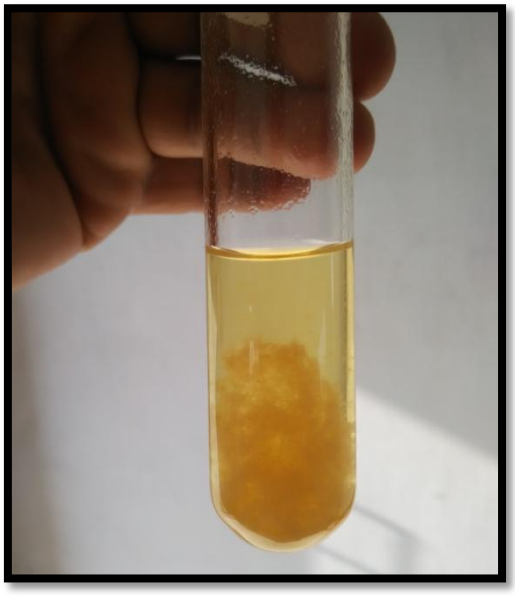


Plate no. 4.12: Pure XOS

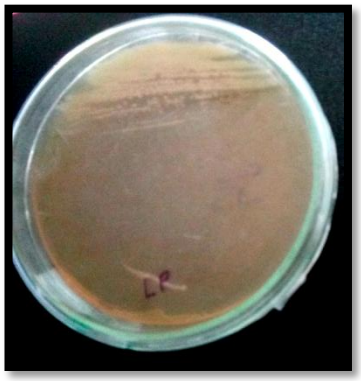


Plate no. 4.13: Petri plate showing growth of *Lactobacillus plantarum*

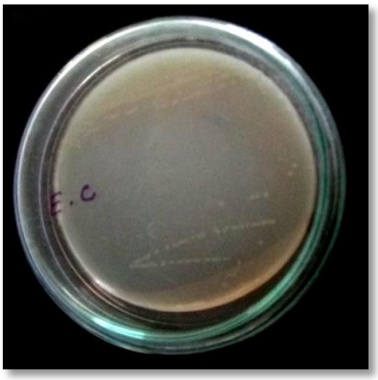


Plate no. 4.14: Petri plate showing growth of *Escherichia coli*

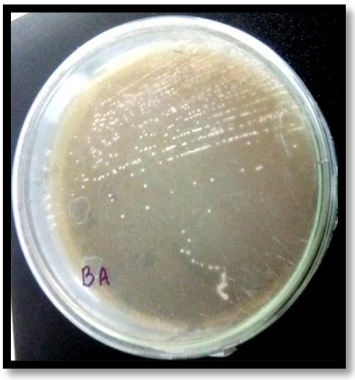


Plate no. 4.15: Petri plate showing growth of *Bifidobacterium adolescentis*

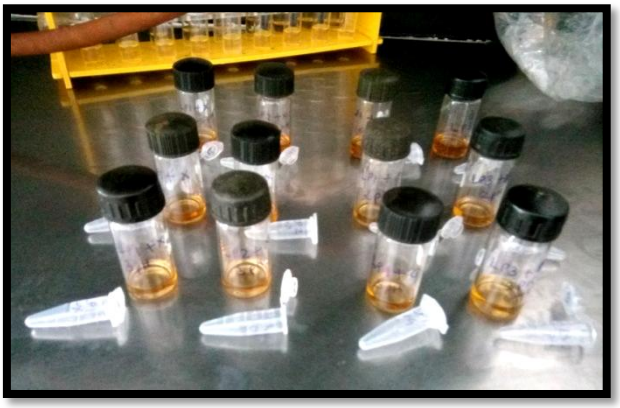


Plate no. 4.16: Glass vials containing bacteria to check bacterial growth in the spectrophotometer

Plate no. 4.17: pH meter

Plate no. 4.18: Sample injection in the HPLC column for SCFA analysis

Plate no. 4.19: HPLC used for analysis

Plate no. 4.20: Glimpse of a panellist performing sensory evaluation of *Black rice kheer*

Plate no. 4.21: Glimpse of panelists performing sensory evaluation of *Gajar ka halwa*

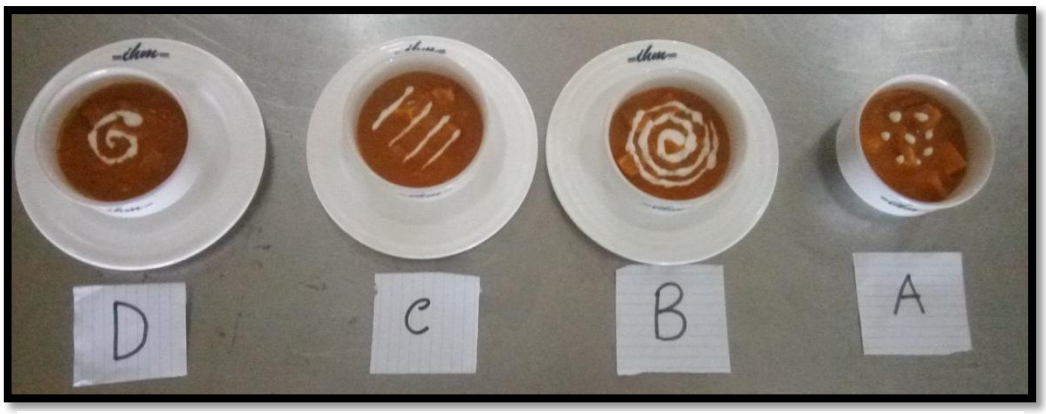


Plate no. 4.22: *Paneer butter masala*

Plate no. 4.23: *Black rice kheer*

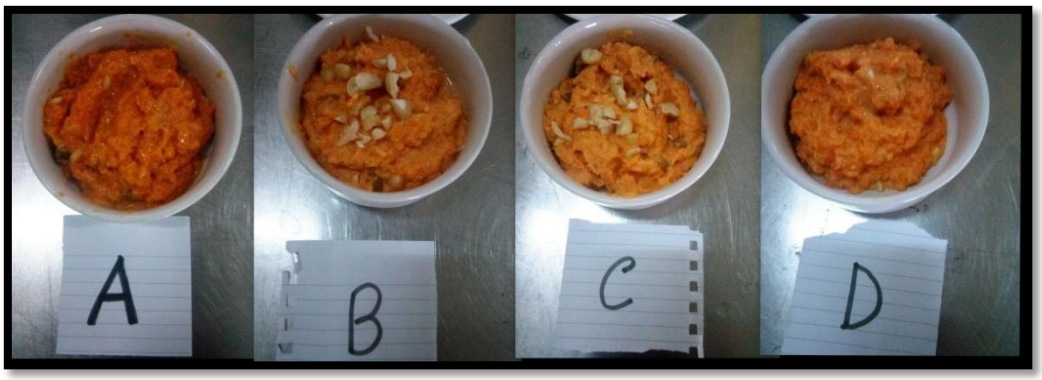


Plate no. 4.24: *Gajar ka halwa*

Plate no. 4.25: *Prawn patia*