CHAPTER 6

SUMMARY AND CONCLUSIONS

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 emerging evidence that functional foods ingredients can have an impact on a number of gut related diseases and dysfunctions (Aachary et al, 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). Several prebiotics such as FOS, (Galactooligosaccharide) GOS, and inulin have been recently recognized for their health promoting abilities. XOS is yet another emerging prebiotic that needs to be explored for its prebiotic potential especially in terms of its acid tolerance, bile resistance and ability to get fermented by the probiotic bacteria.

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 has proven technological benefits in terms of its miscibility and organoleptic qualities.

Therefore, this study was undertaken to study **"Extraction of Xylooligosaccharides (XOS) from agricultural waste, determining its prebiotic properties and organoleptic qualities of Indian traditional foods upon its addition".**

The study is divided into three 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.

The results and major highlights of all the phases of the study are summarized below-

6.1. Phase I

This phase of the research was carried out to extract xylooligosaccharide from selected agricultural wastes, namely, corn cob, orange peels, green banana peels and green pea shells. 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.

Salient features of Phase I

6.1.1. Determination of Xylan in selected agro waste

- Crude xylan yield from corn cob, orange peel, green banana peel and green pea shells using 4% sodium hydroxide (NaOH) was 9.60 g (16.0%), 7.50 g (12.5%), 5.40 g (9.0%), and 4.20 g (7.0%), respectively.

6.1.2. Enzymatic hydrolysis of Xylan

- XOS obtained from the xylan of 60g corn cob, green banana peel, orange peel and green pea shells were 1.8g (18.75%), 1.01g (18.70%), 1.41g (18.80%) and 0.79g

(18.80%) respectively at ($p \leq 0.01$) with an optimal condition of 12h incubation time, pH 5.4 at 40°C.

- Therefore, XOS obtained from 100g dry powdered samples of corncob, green banana peel, orange peel and green pea shells were 3g (18.75%), 1.68g (18.66%), 2.35 (18.80%), and 1.31g (18.71%) respectively.

Table 6.1: XOS obtained from 60 g dried powder

Name of the sample	Weight of whole Raw product (kg)	Weight of the fresh peels (g)	Weight of the dried powder (g)	Xylan obtained (g) from 60 g of dried powder	XOS obtained (g)
Corncob	N/A	N/A	N/A	9.6	1.8
Oranges	2	350	115	7.5	1.41
Green bananas	3	350	74	5.4	1.01
Green peas	2	630	121	4.2	0.79

Table 6.1.1: XOS obtained from 100 g dried powder

Name of the sample	Xylan obtained from 100 g of dried samples (g)	XOS obtained from the xylan (g)
Corncob	16	3g (18.75%)
Orange peels	12.5	2.35 (18.80%)
Green banana peels	9	1.68g (18.66%)
Green pea shells	7	1.31g (18.71%)

6.1.3. Concentration of XOS among all the agro waste samples

- Concentration of XOS obtained from the agro wastes was different.
- Concentration of XOS obtained from corn cob, green banana peels, orange peels and green pea shells was 79.41 mg/ml, 73.50 mg/ml, 74.73 mg/ml and 71.94 mg/ml respectively.

6.2 PHASE II

In this phase, prebiotic properties of XOS in terms of acid tolerance, bile resistance and fermentability of XOS to produce short chain fatty acids (SCFA) were determined using HPLC analysis; growth of the selected bacterial strains was determined using spectrophotometer.

Salient Features of Phase II

6.2.1. Bile resistance test of XOS

- 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%.

6.2.2. Acid tolerance test of XOS

- 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.

6.2.3. Prebiotic effect of XOS on the growth of Lactobacillus plantarum (LP), Bifidobacterium adolescentis (BA) and Escherichia coli (E.coli)

- 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%.

- *Bifidobacterium adolescentis* produced 408.6 mMol(↑331%) propionate on its exposure to XOS. *Lactobacillus plantarum* produced 405.62 mMol(↑188%)

propionate on its exposure to XOS. When *Escherichia coli* were exposed to XOS production of propionate reduced 339.55 mMol (↓20%).

6.2.4. SCFA production analysis during fermentation in vitro

- *Bifidobacterium adolescentis* produced 343.28 mMol(↑331%) butyrate on its exposure to XOS. *Lactobacillus plantarum* produced 340.72 mMol (↑188%) of butyrate on its exposure to XOS. When *Escherichia coli* were exposed to XOS production of butyrate reduced 285.22 mMol (↓21%).

- *Bifidobacterium adolescentis* produced 1833.27 mMol(↑35%) acetate on its exposure to XOS. *Lactobacillus plantarum* produced 1883.82 mMol of acetate on its exposure to XOS and produced 0 mMol when exposed to glucose. When *Escherichia coli* were exposed to XOS production of acetate reduced to 324.65 mMol (↓48%).

6.3 PHASE III

In this phase, two food products from main course and two products from dessert which are popular amongst Indians were selected. Organoleptic evaluation of XOS added *Prawn Patia, Paneer Butter masala*, *Black Rice Kheer* and *Gajar Ka Halwa* with 5g, 8g and 10g XOS.

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 and *Prawn patia* samples containing different 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.

Salient Features of Phase II

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

- 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.

- 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 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.

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- 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 ($p \leq 0.001$).

- Most of the panelists found color of *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.

- 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.

It can be concluded that XOS could be extracted from all the four selected agro wastes. The concentration of XOS obtained from corn cob was found to be highest. However, all the four agro wastes yielded XOS. 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 in all the four products up to 10% indicating that many foods may be enriched with XOS as a prebiotic.

Hence, this study has created a strong evidence-based data to prove the prebiotic potential of XOS. However, further studies can be undertaken to demonstrate the clinical efficacy of XOS intake with respect to various non communicable diseases.