CHAPTER 2

REVIEW OF LITERATURE

Xylooligosaccharides is known to be a potential prebiotic with several health benefits; however we need to find sources for its high content. Most of the agricultural wastes which have no or low 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. In perspective to this, the present study was undertaken to extract Xylooligosaccharides (XOS) from agricultural waste, determine its prebiotic properties and organoleptic qualities of Indian traditional foods upon its addition.

This chapter highlights the available review of literature for the study under the following heads.

2.10 Sensory evaluation of food products

2.10.4 Attributes of a food product

2.10.1.1) Appearance

2.10.1.2) Flavour

2.10.1.3) Aroma

2.10.1.4) Texture

2.10.1.5) Sound

2.11. Types of tests

a) Difference tests: a.1) Triangle test a.2) Paired comparison test a.3) Duo-trio test

b) Rating tests: b.1) Ranking test b.2) Single sample test b.3) Two sample difference test b.4) Multiple sample difference test b.5) Hedonic rating test b.6) Numerical scoring test b.7) Composite scoring test

c) Sensitivity tests: c.1) Sensitivity threshold test c.2) Dilution test

d) Descriptive tests

2.12. Selection and screening of Panel Members.

2.13. Training of panellists

2.14. Technological benefits of adding prebiotics in foods

2.15. Future scope of prebiotics

2.15.1. Future scope of XOS as a prebiotic

2.15.2. Prebiotics in food industries

2.1 Agricultural produce in India

India is the largest producer of spices, pulses, milk, tea, cashew and jute; and the second largest producer of wheat, rice, fruits and vegetables, sugarcane, cotton and oilseeds. India is currently the world's fourth largest producer of agro chemicals. India has the largest livestock population of around 535.78 million, which translates to around 31 percent of world population. During 2018-19 crop year, food grain production estimation was 284.95 million tonnes. In 2019-20, Government of India is targeting food grain production of 291.10 million tonnes (IBEF, 2020).

India has two major agricultural seasons: Kharif and Rabi. Kharif season lasts from April to September (summer); rice (paddy) is the season's main crop. Rabi season lasts from October

to March (winter); wheat is the season's main crop. As of September 2019, total area sown with Kharif crops in India reached 92.6 million hectares (IBEF, 2020).

Fig: 2.1 (a) shows Rabi Area Sown in 2018-19 (million hectares) (as on January 31, 2020). Crops sown in Rabi season were wheat, pulses, oilseeds, coarse cereals and rice.

Fig: 2.1 (b) shows Kharif Area Sown in 2019-20 (million hectares) (as on September, 2019). Crops sown were rice, pulses, coarse cereals, oilseeds, sugarcane and cotton.

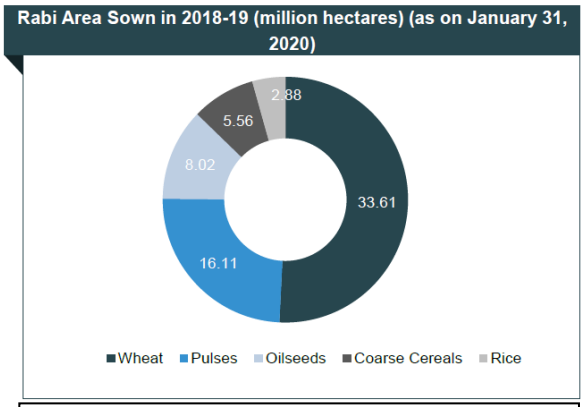

Fig: 2.1 (a) Rabi Area Sown in 2018-19 (million hectares) (as on January 31, 2020)

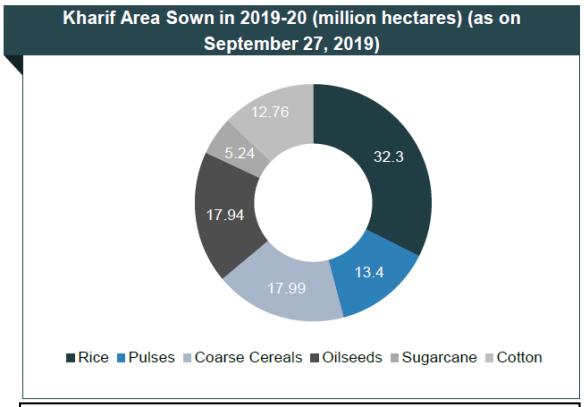

Fig: 2.1 (b) Kharif Area Sown in 2019-20 (million hectares) (as on September, 2019)

(IBEF, 2020).

India ranked second in global production of fruits and vegetables and is a leading exporter of mangoes and bananas. India was the ninth largest exporter of agricultural products in 2017. Marine Products, Buffalo Meat and rice are largest agricultural export items in terms of value. Other major export items are spices, cotton, oil products and sugar (IBEF, 2020).

Marine product exports reached US$ 4.8 billion in FY20, followed by Basmati rice at US$ 2.53 billion and buffalo meat at US$ 2.25 million. Tea exports from India reached to 270.27 million kgs in FY19 while coffee exports stood at 282.87 million kgs in FY19. During April-December 2019, tea export stood at US$ 600 million. The coffee export stood at US$ 667.47 million during April 2019-February 2020 (IBEF, 2020).

2.1.1 Production of Banana in India

Table: 2.1.1 shows top 10 Banana producing states of India in the year 2017-18. Andhra Pradesh tops the list and Gujarat is at second position in Banana production in the country (Source: National Horticulture Board, APEDA).

Indian Production of BANANA

Production(000 Tonnes)

		2017-18	
Sr No.	**State**	**Production**	**Share(%)**
1	Andhra Pradesh	5,003.07	16.27
2	Gujarat	4,472.32	14.54
3	Maharashtra	4,209.27	13.69
4	Tamil Nadu	3,205.04	10.42
5	Uttar Pradesh	3,172.33	10.31
6	Karnataka	2,328.90	7.57
7	Madhya Pradesh	1,834.03	5.96
8	Bihar	1,396.39	4.54
9	West Bengal	1,200.00	3.90
10	Kerala	1,119.16	3.64
	Page Total	**27,940.51**	

(Source: National Horticulture Board, APEDA).

2.1.2 International Production of Banana

Table: 2.1.2 shows top 10 Banana producing countries in 2018. India tops the list and China is at the second rank in Banana production globally (Source: FAO, APEDA).

International Production : Bananas

Production in (000) MT

		2018	
Sr No.	**Country**	**Production**	**Share(%)**
1	India	30,808.00	26.62
2	China P Rp	11,221.70	9.70
3	Indonesia	7,264.38	6.28
4	Brazil	6,752.17	5.83
5	Ecuador	6,505.64	5.62
6	Philippines	6,144.37	5.31
7	Guatemala	4,026.55	3.48
8	Colombia	3,707.15	3.20
9	Angola	3,492.18	3.02
10	Tanzania Rep	3,469.09	3.00
	Total	**83,391.23**	

(Source: FAO, APEDA).

2.1.3 Production of Mandarin (M.Orang, Kinnow, Orange) in India

Table: 2.1.3 shows top 10 Mandarin producing states of India in the year 2017-18. Madhya Pradesh tops the list and Punjab is at second position in Mandarin production in the country (Source: National Horticulture Board, APEDA).

Indian Production of MANDARIN(M.ORANG,KINNOW,ORANGE)

Production(000 Tonnes)

		2017-18	
Sr No.	**State**	**Production**	**Share(%)**
1	Madhya Pradesh	2,103.64	41.24
2	Punjab	1,208.42	23.69
3	Maharashtra	797.95	15.64
4	Rajasthan	317.68	6.23
5	Assam	203.72	3.99
6	Karnataka	79.07	1.55
7	Arunachal Pradesh	69.74	1.37
8	Nagaland	47.33	0.93
9	Meghalaya	45.24	0.89
10	Mizoram	44.02	0.86

(Source: National Horticulture Board, APEDA).

2.1.4 International Production of Oranges

Table: 2.1.4 shows top 10 Orange producing countries in 2018. Brazil tops the list and India is at the third rank in Orange production globally (Source: FAO, APEDA).

International Production : Oranges

Production in (000) MT

		2018	
Sr No.	**Country**	**Production**	**Share(%)**
1	Brazil	16,713.53	22.17
2	China P Rp	9,103.91	12.07
3	India	8,367.00	11.10
4	U S A	4,833.48	6.41
5	Mexico	4,737.99	6.28
6	Spain	3,639.85	4.83
7	Egypt A Rp	3,246.48	4.31
8	Indonesia	2,510.44	3.33
9	Turkey	1,900.00	2.52
10	Iran	1,889.25	2.51

(Source: FAO, APEDA).

2.1.5 Production of Peas in India

Table: 2.1.5 shows top 10 Pea producing states of India in the year 2017-18. Uttar Pradesh tops the list and Madhya Pradesh is at second position in Pea production in the country (Source: National Horticulture Board, APEDA).

Indian Production of PEAS

Production(000 Tonnes)

		2017-18	
Sr No.	**State**	**Production**	**Share(%)**
1	Uttar Pradesh	2,511.38	46.37
2	Madhya Pradesh	961.55	17.76
3	Punjab	394.00	7.28
4	Jharkhand	347.14	6.41
5	Himachal Pradesh	294.96	5.45
6	West Bengal	144.25	2.66
7	Chattisgarh	137.16	2.53
8	Haryana	135.15	2.50
9	Uttarakhand	93.40	1.72
10	Manipur	84.32	1.56

(Source: National Horticulture Board, APEDA).

2.1.6 International Production of Peas

Table: 2.1.6 shows top 10 Pea producing countries in 2018. China tops the list and India is at the second rank in Pea production globally (Source: FAO, APEDA).

International Production : Peas, Green

Production in (000) MT

		2018	
Sr No.	**Country**	**Production**	**Share(%)**
1	China P Rp	12,960.84	61.07
2	India	5,430.00	25.59
3	France	251.08	1.18
4	U S A	230.05	1.08
5	Egypt A Rp	202.76	0.96
6	Algeria	186.20	0.88
7	Pakistan Ir	178.23	0.84
8	Peru	135.91	0.64
9	Morocco	123.22	0.58
10	U K	119.17	0.56

(Source: FAO, APEDA).

2.1.7 Production of Maize in India

Table: 2.1.7 shows top 10 Maize producing states of India in the year 2017-18. Karnataka is at second position in Maize production in the country (Source: National Horticulture Board, APEDA).

Indian Production of Maize

Production(000 Tonnes)

		2017-18	
Sr No.	**State**	**Production**	**Share(%)**
1	Others	3,910.00	13.61
2	Karnataka	3,550.00	12.36
3	Madhya Pradesh	3,540.00	12.33
4	Maharashtra	3,540.00	12.33
5	Tamil Nadu	2,640.00	9.19
6	Telangana	2,570.00	8.95
7	Bihar	2,420.00	8.43
8	Andhra Pradesh	2,300.00	8.01
9	Rajasthan	1,640.00	5.71
10	Uttar Pradesh	1,480.00	5.15

(Source: National Horticulture Board, APEDA).

2.1.8 International Production of Maize

Table: 2.1.8 shows top 10 Maize producing countries in 2018. USA tops the list and India is at the seventh rank in Maize production globally (Source: FAO, APEDA).

International Production : Maize

Production in (000) MT

		2018	
Sr No.	**Country**	**Production**	**Share(%)**
1	U S A	3,92,450.84	34.20
2	China P Rp	2,57,173.90	22.41
3	Brazil	82,288.30	7.17
4	Argentina	43,462.32	3.79
5	Ukraine	35,801.05	3.12
6	Indonesia	30,253.94	2.64
7	India	27,820.00	2.42
8	Mexico	27,169.98	2.37
9	Romania	18,663.94	1.63
10	Canada	13,884.80	1.21

(Source: FAO, APEDA).

Amongst all horticultural crops, the most utilized ones are fruits and vegetables. They are consumed raw, processed, partially processed etc. As the population is growing and diet habits are changing, the production and processing of horticultural crops, especially fruits and vegetables have increased significantly to fulfill the increasing demands. With this increase, losses and wastes in the fresh and processing industries are becoming a serious nutritional, economical, and environmental problem. Fruits and vegetables processing produce

significant wastes of by-products, which is about 25% to 30% of a whole commodity group (Sagar et al, 2018).

These wastes are mainly composed of seed, skin, rind, and pomace, containing good sources of potentially valuable bioactive compounds, such as carotenoids, polyphenols, dietary fibers, vitamins, enzymes, and oils, among others. In different industries including the food industry, these phytochemicals can be utilized for the development of functional or enriched foods, the health industry for medicines and pharmaceuticals, and the textile industry, among others. An important step towards sustainable development is to use the wastes for the production of various crucial bioactive components (Sagar et al, 2018).

2.2 Agro waste from different edible plants.

2.2.1 Agro-Industrial wastes

Agro-industries are not just fruits and vegetables, crops, such as rice, sugarcane, jute, tea and coffee, but also forest products (non-edible oilseeds, wood, etc). These wastes are available in huge quantities at processing sites whereas, animal wastes and crop residues are available in a scattered way (Pal et al, 2014).

It is also known that agro-industrial wastes are rich in bioactive compounds and nutrient composition. These wastes are composed of sugars, minerals, and proteins; hence, they should be considered as “raw material” instead of “wastes” for other industrial processes. The presence of these nutrients in the residues offers suitable conditions for the prolific growth of microorganisms. The microorganisms have potential to reuse the waste as raw materials for their growth through fermentation processes. The use of agricultural and agro-based industry wastes as raw materials can help to reduce the production cost and contribute in recycling of waste as well to make the environment eco-friendly (Sadh et al, 2018).

2.2.2 Rice Milling Industry wastes

Rice husk and rice bran

The largest product of the rice milling industry is rice husk which comprises 20% to 25% of paddy. This paddy in turn yields about 5% to 7% bran. The availability of rice husk is about 15 MT annually. A paddy husk sample contains 42.6% cellulose, 20.1% Iignin,

18.6% pentosans and 18.7% ash. It is a poor source of manure and its N content varies from 0.3 to 0.4 per cent, P_2O_5 0.2 to 0.3 per cent and K_2O 0.3 to 0.5 per cent. In certain areas, there is a problem of its disposal. It is used as fuel and to improve physical conditions of saline and alkaline soils. It can also be used as bedding material for animals and in composting. Rice bran yield is about 2.5 MT annually and has limited scope as fertilizer since this is exploited for production of rice bran oil (Pal et al, 2014).

2.2.3 Sugar milling industry wastes

The major by-products of sugar industry are bagasse, pith and molasses are presumed a multi ingredient and vital source of macro and micro nutrients, matrix for microbes, source of phytosteroides and protectinstoriodes.

Bagasse

Indian sugarcane contains 12% to 17% fibre and 33% bagasse. Annually, about 5.3 million tonnes of dry bagasse is produced which is almost entirely used as fuel in boilers of sugar factories. Some of the studies have shown that bagasse is a valuable material for production of pulp paper, boards etc. It is estimated that about 1.4 million tonnes of organic manure per year can be produced from this by product. However, a portion of bagasse could be utilized as both for fuel and manure if it is processed through biogas plants. Xylooligosaccharides can also be produced from baggase. The nitrogen and P_2O_5 percent of bagasse is approximately 0.25% and 0.12% and compost produced out of it will have nitrogen of 1.4% and 0.4% of P_2O_5. (Pal et al, 2014).

2.2.4 Vegetable and fruit processing industry wastes

Vegetable and fruit wastes

India produces around 33 million tonnes of fruits and 50 million tonnes of vegetables annually. It is estimated that roughly 10% to 15 % of total produce is available either as residues or bio-degradable wastes for recycling in agriculture. Around 5 million tonnes of solid wastes are produced from fruits and vegetables processing. Most of these wastes are lignocellulosic in nature and contain macro and micro-nutrients. If the wastes of plantation crops are managed, it will provide 165 thousand tonnes of $N+P_2O_5+K_2O$, which will definitely provide help for using indigenous materials for maintaining sustainable agriculture. There is a wide scope for utilization of these wastes as fertilizer. The total

quantity available from this industry is more than 25,000 tonnes from mango, pineapple, citrus fruits, apples, green peas, tomato etc annually. It is estimated that about 10,000 tonnes of compost could be produced out of these wastes (Pal et al, 2014).

To reduce the challenges of overproduced agricultural by-products or agricultural wastes, attempts are being made worldwide for generation of value added products such as xylan, xylooligosaccharides (XOS), xylitol through application of bio refinery approach keeping in mind the environmental and economic concerns (Ruzene et al, 2008, Aachary et al, 2009, Gullon et al, 2010, Teng et al, 2010 and Samanta et al, 2014). Like other plant biomass, corn cobs are composed of mainly cellulose, hemicelluloses and lignin, and offers great promise as precursors for several biomolecules having industrial significance and health-promoting prospects such as prebiotic XOS (Samanta et al, 2014).

2.3 Prebiotics, their types and mechanism of action

'A non digestible compound through its metabolization by microorganisms in the gut modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiologic effect on the host' (Bindels et al, 2015).

'Prebiotics is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host health.' (Gibson et al, 2010).

These modifications take place at the individual level strains and species and cannot be easily predicted from before. The gut environment, especially pH, plays a major role to determine the outcome of interspecies competition. The development of prebiotics intended to benefit human health has to take account of the highly individual species profiles that may result both for reasons of efficacy and of safety (Markowiak et al, 2017).

There are a few criteria to classify a food ingredient as a prebiotic:
(1) It should be resistant to gastric acidity, hydrolysis by mammalian enzymes and GI absorption;
(2) It should be fermented by the indigenous intestinal microflora;
(3) The growth and/or activity of the intestinal bacteria should be selectively stimulated by this compound and improve host's health (such as bifidobacteria, lactobacilli) (Gibson et al, 2006, Davari et al, 2019).

There are two types of prebiotics, i.e., those occurring naturally in plants such as banana, asparagus, beans, and cereals, and those synthesized from enzymatic digestion of polysaccharides, such as starch (Thammarutwasik et al, 2009).

Naturally occurring prebiotics	Synthesized prebiotics
Galactooligosaccharides Fructooligosaccharides and Inulin Soybean oligosaccharides (SOS)	Lactosucrose (LS) Lactulose Isomaltooligosaccharide (IMO) Glucooligosaccharides Xylooligosaccharides (XOS) FOS and Inulin

2.4 Types of Prebiotics

FOS, GOS and inulin have long been considered as prebiotics. The following prebiotics have sufficient evidence that they promote digestive health for consumers.

2.4.1 Beta-glucan

Beta-glucans are composed of linear D-glucopyranosyl units with a mixture of β-(1,3) and β-(1,4) glycosidic linkages. They are the soluble compounds found in the endosperm cell walls of cereals and foods like mushrooms, algae, and other marine plants. Two of the highest sources of beta-glucans in the diet are oats and barley. Beta-glucans has a wide range of impacts on GI health of host because of their variation in length and branching (Carlson et al, 2018).

2.4.2 Fructooligosaccharides (FOS), oligofructose, and inulin

In a wide range of foods, fructan compounds (β[2,1]-fructans) are found. Inulin has a degree of polymerization (DP) between 3 and 60 fructan monomers. From the chemical degradation of these products with endoglycosidase enzymes, yielding a product with a DP of 2–20, oligofructose is made. FOS contain 2 and 4 β(2,1)-linked fructosyl units produced from the transfructosylation of sucrose. All of these compounds exert strong bifidogenic effects, irrespective of the length of polymerization influencing these effects (Carlson et al, 2018).

2.4.3 Galactooligosaccharides (GOS)

GOS are derived from the enzymatic glycosylation of primary lactose, primarily using β-galactosidases to catalyze various transgalactosylation reactions. GOS are composed of 2–10 molecules of galactose and 1 molecule of glucose, primarily synthesized from enzymatic activity. The

prebiotic capacity of GOS in many clinical studies is influenced by the purity, degree of polymerization, type, and dosage of GOS (Carlson et al, 2018).

2.4.4 Isomaltooligosaccharides

Isomaltooligosaccharides are made from enzymatic treatment of cornstarch with α-amylase, pullulanase, and α-glucosidase These are glucose monomers linked by α(1,6)- glucosidic linkages and its primary components are isomaltose, isomaltotriose, and panose. Effective doses range from 5–10 g/d for most individuals depending on the final DP. Adults could tolerate doses as high as 30 g/d with only mild GI side effects being mentioned (Carlson et al, 2018).

2.4.5 Guar gum

Guar gum is composed of high molecular weight polysaccharides ([1,4]-linked β-D-mannopyranosyl units with [1,6]-linked α-D-galactopyranosyl side chain residues). It is a gel-forming galactomannan made from the endosperm of the plant Cyamopsis tetragonolobus. It is commonly used in dairy, bakery, cereal, and meat products (Carlson et al, 2018).

2.4.6 Lactulose

Lactulose is composed of galactosyl β(1,4) fructose derived from the primary and secondary isomerization of lactose. It is a disaccharide, not digestible by mammalian enzymes, nor hydrolyzed or absorbed in the small intestine. It is not found naturally in foods, limited clinical studies have consistently shown beneficial effects due to the fermentation of these compounds (Carlson et al, 2018).

2.4.7 Resistant starch (RS) and maltodextrin

Resistant starches escape digestion in the upper GI tract. They are a broad categorization of many classes of starches formed under a variety of conditions. Resistant maltodextrin is produced by treating cornstarch with numerous acid, enzymatic, and heating processes. It is a highly water-soluble, low-viscosity dextrin that is used in a variety of applications. Some resistant starches are naturally found in foods, whereas others are artificial (Carlson et al, 2018).

2.4.8 Xylooligosaccharides (XOS) and arabinooligosaccharides

Xylooligosaccharides are composed of between 2 and 10 xylose monomers linked with β 1,4 bonds with DP ≤20. XOSs are commonly found in dairy products, cereals, bars, sports drinks, and isotonic beverages. Japan has approved XOS as an ingredient for “Food for Specified

Health Use" since 1991, and according to regulation, is expected to have a specific effect on health. Japan is responsible for nearly half of the production and consumption of XOS worldwide (Carlson J L. et al, 2018). In human intervention studies, doses of ≤12 g/d have shown to be well tolerated (XOS GRAS Notice, January 2013).

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 bagasse, wheat straw, cotton stalks, orange peels, mango peels etc (Moure et al, 2006).

2.4.8.1 Acid and thermal stability of XOS

Xylooligosaccharides are stable after heating to 100℃ under acidic conditions (pH=2.5-8) that cover the pH values of the vast majority of food systems (Courtin et al, 2009; Vazquez et al, 2000).

In food processing, XOS show advantages over inulin in terms of resistance to both acidity and heat, allowing their utilization in low-pH juices (Vazquez et al, 2000).

2.5 Importance of prebiotics in health

Recent studies suggested that the gut microbiota could be manipulated using diet, prebiotics, and probiotics in order to maintain health. Diets which contain nutrients that are fermentable by intestinal bacteria may be used for stimulating the growth of beneficial bacteria (Lin et al, 2014).

To stimulate the growth and activity of beneficial bacteria in the gut that confers a health benefit on the host is the main aim of prebiotics. Mechanisms which include antagonism and competition for epithelial adhesion and for nutrients, the intestinal microbiota acts as a barrier for pathogens. SCFAs, namely: acetic acid, butyric acid, and propionic acid are end products of carbohydrate metabolism. These are subsequently used by the host as a source of energy. *Bifidobacterium* or *Lactobacillus* may produce some compounds inhibiting the development of GI pathogens and cause a reduction in the intestinal pH as output of the fermentation of carbohydrates. *Bifidobacterium* shows tolerance to the produced SCFAs and reduced pH. Therefore, administration of prebiotics may inhibit the development of pathogens due to their favourable effect on the development of beneficial intestinal bacteria (Markowiak et al, 2017).

Several diseases are associated with dysbiosis of the gut microbiota suggesting that intestinal bacteria could be used as a signature for disease conditions. An important therapeutic strategy to prevent and treat human diseases could be by modifying the gut microbiota using prebiotics and probiotics (Lin et al, 2014).

2.6 Mechanism of action of prebiotics

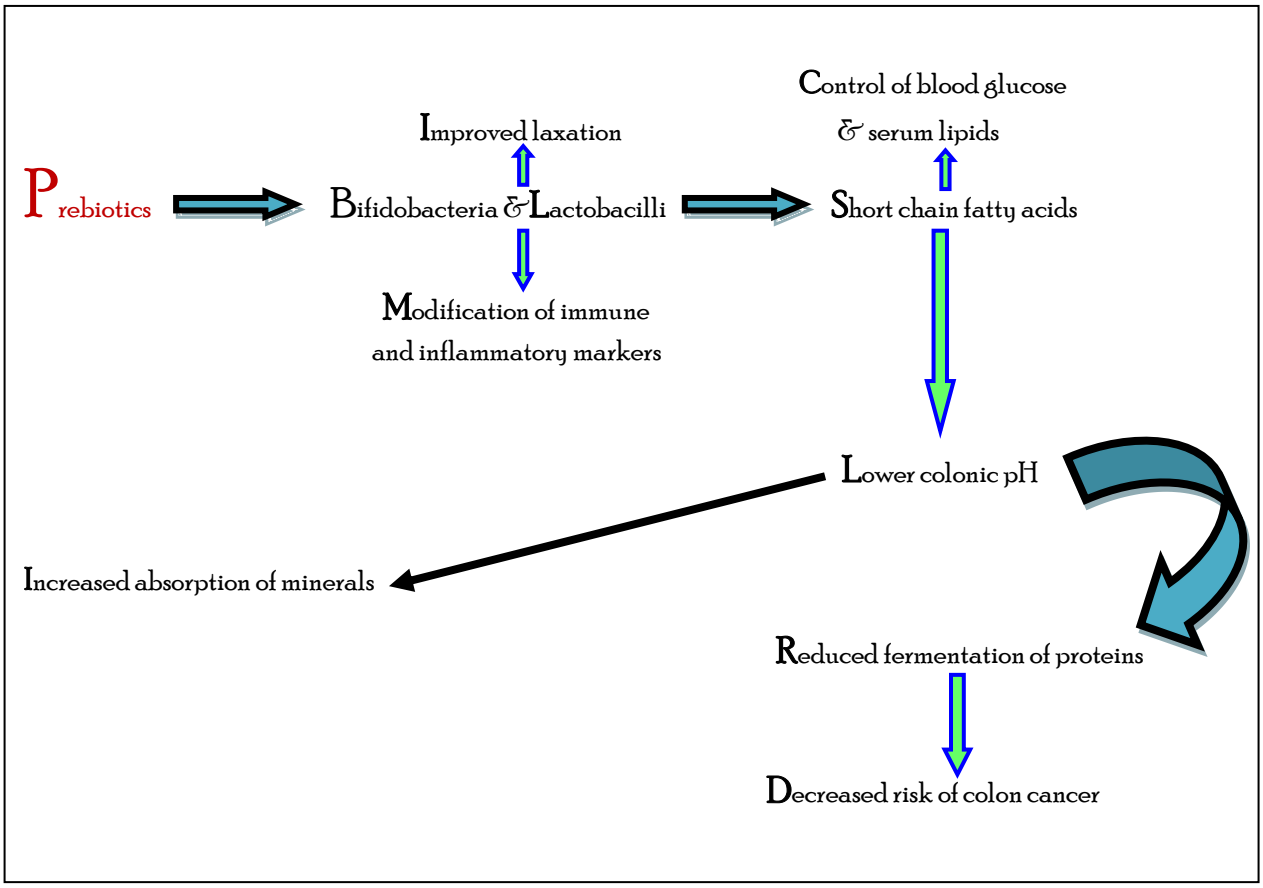

Fig 2.2: Schematic diagram of mechanism of action of prebiotics.
Adopted from Saville BA et al, 2018.

2.4.1. Mechanism of action of prebiotics on lowering glucose and insulin levels

It has been found to be associated with the SCFAs, especially propionate. A significant reduction in post-prandial glucose concentrations was observed following both acute and chronic intakes of propionate-enriched bread.

The effects were attributed to the actions of OFS on the secretion of the gut hormones, glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1(GLP-1).These hormones are secreted from the small intestine (GIP) and the terminal ileum and colon (GLP-1) and contribute to the secretion of insulin following a meal in the presence of raised glucose levels. (Gibson et al, 2000).

2.6.2 Mechanism of lipid lowering by prebiotics

Prebiotics have been shown to be an ideal substrate for the health-promoting bacteria in the colon, notably *bifidobacteria* and *lactobacilli*. Gases (H_2S, CO_2, H_2 and CH4), lactate and SCFAs (acetate, butyrate and propionate) are produced during the fermentation process. The SCFAs, acetate and propionate enter the portal blood stream where they are utilised by the liver and acetate is converted to acetyl CoA in the liver and acts as a lipogenic substrate for *de novo* lipogenesis, whereas propionate has been reported to inhibit lipid synthesis. Butyrate is taken up by the large intestinal cells (colonocytes) and protects against tumour formation in the gut. The SCFAs that are produced during the fermentation process is dependent on the microflora which can be stimulated by the prebiotic. Inulin has been shown to increase both acetate and butyrate levels, whereas synthetically produced prebiotics, for example galacto-oligosaccharides, increase the production of acetate and propionate and xylooligosaccharides increase acetate only. Inulin and FOS have been extensively studied to determine the mechanism of action of prebiotics in animals. Early *in vitro* studies using isolated rat hepatocytes suggested that the hypolipaemic action of FOS was associated with the inhibition of *de novo* cholesterol synthesis by the SCFA propionate following impairment of acetate

utilisation by the liver for *de novo* lipogenesis. This is in agreement with human studies in which rectal infusions of acetate and propionate resulted in propionate inhibiting the incorporation of acetate into TAGs released from the liver. Research evidence has suggested that the TAG lowering effect of FOS occurs via reduction in VLDL, TAG secretion from the liver due to the reduction in activity of all lipogenic enzymes (acetyl-CoA carboxylase, fatty acid synthase, malic enzyme, ATP citrate lyase and glucose-6-phosphate dehydrogenase), and in the case of fatty acid synthase, via modification of lipogenic gene. (Gibson et al, 2000)
The main physiological and patho-physiological targets for prebiotic effects, i.e. effects associated with a selective stimulation of growth and/or activity (ies) of one or a limited number of gut microorganisms are (Roberfroid et al, 2010):

- Improvement and/or stabilization of gut microbiota composition Improvement of intestinal functions (stool bulking, stool regularity, stool consistency)
- Increase in mineral absorption and improvement of bone health (bone Ca content, bone mineral density)
- Modulation of gastro-intestinal peptides production, energy metabolism and satiety Initiation (after birth) and regulation/modulation of immune functions Improvement of intestinal barrier functions, reduction of metabolic endotoxemia
- Reduction of risk of intestinal infections and tentatively Reduction of risk of obesity, type 2 diabetes, metabolic syndrome, etc.
- Reduction of risk and/or improvement in the management of intestinal inflammation.
- Reduction of risk of colon cancer.

Although probiotic and prebiotic approaches are likely to share common mechanism of action, as their effect is impacted through increase in beneficial colonic bacteria, they differ in composition and metabolism.

2.6.1 Effect prebiotic on glycemia, lipemia, hypertension and hs-CRP

An intervention study assessed the effect of FOS supplementation in type 2 diabetic adults on their lipemic, biophysical parameters and gut microflora parameters. The experimental group supplemented with 10 g of FOS for eight weeks resulted in a significant reduction ($p<0.05$) in systolic blood pressure and an appreciable reduction in serum TC, TG and LDL levels by 10%, 4.9% and 7.8% respectively (Mahendra et al, 2013).

Inulin is thought to share many of the properties of soluble dietary fibers, such as the ability to lower blood lipids and stabilize blood glucose. Additionally, inulin has been shown to enhance the growth of *bifidobacteria* and *lactobacilli* and enhance the gut environment. Randomly twelve men were assigned to two controlled diets that differed only in that the control diet contained one pint of vanilla ice cream made with sucrose while the inulin containing diet was supplemented with one pint of vanilla ice cream made with 20 grams of inulin. Subjects consumed each controlled diet for three weeks. Daily intake of 20 g of inulin significantly reduced serum triglycerides by 40 mg/dL($p=0.05$). A trend toward a reduction in serum cholesterol was also observed and trends toward short chain fatty acid (SCFA) profile changes were observed after inulin administration. (Causey, 2000)

Another study reported in a reduction in TG, LDL and TC by 22.8%, 16.5% and 15.4% respectively when the young obese subjects were supplemented with 20 g of FOS for 3 months (Jain et al, 2014).

A randomized controlled clinical trial was undertaken in Iran to see if prebiotic inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus. The diabetic female subjects were given 10 g inulin for 8 weeks and they exhibited a significant decrease in FBS (8.5%), HbA1c (10.4%), fasting insulin (34.3%) and (39.5%) hsCRP. Hence, it concluded that inulin supplementation seems to be able to modulate inflammation and metabolic endotoxemia in women with type 2 diabetes (Dehghan, 2014).

A randomized controlled trial study on the effect on the blood lipid profile of soy foods combined with a prebiotic enrolled twenty-three hyper lipidemic adults (11 male, 12 female) who completed three 4-week diet intervention phases—a low-fat dairy diet and 10 g/d prebiotic (oligofructose-enriched inulin, a fermentable carbohydrate), a soy food–containing diet (30 g/d soy protein, 61 mg/d isoflavones from soy foods) and 10 g/d placebo (maltodextrin), and a soy food–containing diet with 10 g/d prebiotic. Intake of soy plus prebiotic resulted in greater reductions in LDL-C (P = .042) and in ratio of LDL-C to high-density lipoprotein cholesterol (P = .041) compared with prebiotic. In addition, high-density lipoprotein cholesterol was significantly increased on soy plus prebiotic compared with prebiotic (P = .029). (Wong et al, 2010).

2.7 Prebiotic potential of XOS.

XOS have been identified as a potential novel prebiotic and its benefits include enhancement of the biological availability of minerals such as calcium, iron etc. by improving its absorption, reduction in the risk of colon cancer, cytotoxic effects on human leukemia cells, positive effects on the diabetes and improvement in bowel function and gut health (Jain et al, 2015)..

XOS is not digested by the enzymes secreted by vertebrate's own organelles and reaches hindgut after retaining its structural integrity due to the presence of β-1,4-xylosidic bonds. Hindgut is the home of microbial habitat in our body which contains both beneficial and harmful microflora (Samanta et al, 2015).

A comparative study suggested that 4g of XOS for 3 weeks per day improves the gut microbiota in the elderly people > 65 years. Several reports were found on the utilization of XOS by beneficial bacteria like *bifidobacteria* and other *lactic acid bacteria*. These bacteria produce SCFAs by fermenting xylooligosaccharides resulting into many beneficial effects (Jain et al, 2015).

At the beginning of fermentation, lactate is produced as an intermediary product of XOS and is further metabolized into acetate, butyrate and propionate by intestinal microflora. Consumption of prebiotics is related to decrease in fecal and caecal pH; reduction in the growth of harmful microflora such as *C. perfringens*, *E. coli* etc. by switching the growth stimulation of beneficial gut flora (Samanta et al, 2015).

Effects of XOS on the intestinal microbiota, GI function and nutritional parameters in elderly patients found that a daily supplementation of 4g XOS in a three-week period promoted intestinal health and depicted no adverse effects on the nutritional status of the elderly patients (Chung et al, 2007).

In an intervention study, supplementation of XOS, 8 g/day was given to healthy adults (25–65 years) for 21 days. Supplementation of XOS increased mean bowel movements per day, but did not alter the symptoms of abdominal pain or flatulence, bloating, or the incidence of any reported adverse events. XOS supplementation significantly increased participant-reported vitality and happiness. It significantly increased faecal *bifidobacterial* counts and fasting plasma HDL concentrations. It also demonstrated that XOS induce bifidogenesis, improve aspects of the plasma lipid profile and modulate the markers of immune function in healthy adults. (Childs et al, 2014).

An in vitro study showed XOS digestibility in the GI tract and its effect on absorption of bile acids compared to the effects of FOS and isomaltooligosaccharides (IOS). HPLC analyses depicted the hydrolysis of FOS, IOS and XOS products after 4 hours of digestion. Part of the FOS and most of the IOS was digested by the intestinal juice, while XOS did not undergo digestion by any digestive enzyme. In vitro experiments confirmed delay in the effects of XOS on bile acid absorption compared with IOS and FOS (de Sousa et al, 2011).

Another study showed the fermentation of two new hard-wood derived xylan, xylooligosaccharides and a commercial XOS preparation in a human colon simulator (EnteromixR) by human microbiota. The XOS was selectively fermented by *Bifidobacterium lactis* strains and as a prebiotic reference, FOS was used. In the colonic model, XOS was more efficient than FOS in increasing the numbers of *B. lactis*. A combination of XOS and *B. lactis* might be possible to formulate strain-specific synbiotic product (Makelainen et al, 2010).

Moreover, XOS have immense potential in improving the quality of several foods by food flavour modification and improvement in their physiochemical characteristics because of its stability and wide range of pH and temperature. It could be used in soft drinks, soy milk, cocoa drinks, tea, nutritive preparations, dairy products like milk, milk powder, yogurt, candies, jellies, jam and honey products to formulate health foods or value added food products. XOS production cost is a big hurdle in incorporating them into daily meals, which could be reduced by some economical substrates and efficient processes (Jain et al, 2015).

A study analysed the prebiotic activity of XOS obtained from corncob and reagent grade xylan. They were tested in *L. acidophilus, L. brevis, L. plantarum, L. rhamnosus* cultures and in a co-culture with *E. coli* as a challenge microorganism to prove the bacteriostatic activity of lactobacilli strains. XOS stimulated the growth of *L. brevis* and *L. plantarum*: these microorganisms grew faster than other lactobacilli strains. *L. acidophilus* grew better in the presence of XOS. In the co-culture in presence of both XOS, *E. coli* did not grow; lactobacilli colonies appeared in MRS agar (Pedraza et al, 2014).

A recent study reported the effects of XOS incorporated rice porridge consumption on the intestinal tract's ecosystem of 20 healthy subjects in 6-week intervention trial, 10 subjects received XOS incorporated rice porridge and the rest received placebo rice porridge. They reported that 6 week daily consumption of the XOS incorporated rice porridge significantly increased fecal bacterial counts of *Lactobacillus* spp. and *Bifidobacterium* spp. decreased the growth of *Clostridium perfringens* compared to that of placebo rice porridge. The study concluded that the intestinal microbiota balance improved after daily consumption of 150 g of rice porridge containing 1.5g XOS for 6 weeks, depicting the prebiotic potential of XOS incorporated foods (Lin et al, 2016).

2.8 Methods of XOS production.

Various methods can be used to extract XOS from xylan rich agricultural wastes. Some of the important ones are *chemical methods*, *auto hydrolysis* at very high temperature and *direct enzymatic hydrolysis* or a *combination of chemical and enzymatic treatments* (Jain et al, 2015). From the several methods of XOS extraction, enzymatic hydrolysis with xylanase does not produce any toxic by-products unlike other methods (Thakuria et al, 2018).

XOS can also be produced by 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 (Thakuria et al, 2018).

Initially, raw materials used for XOS production were hardwoods (e.g., birchwood, beechwood), corn cobs, straws, bagasse, rice hulls, malt cakes, and bran. In recent years, the functional food market is growing fast and increase in the number of other industrial applications are eventually encouraging identification of cheap and renewable xylan sources instead of hardwood xylan for production of XOS. As a result, xylan rich agricultural

residues such as cotton stalks, tobacco stalks, and wheat straw have also been intensively studied (Qing et al, 2013).

2.8.1 Chemical methods: XOS production includes alkali extraction or treatment of lignocellulosic materials (LCMs) with mineral acid solutions. Strong alkaline solutions like KOH, NaOH, Ca $(OH)_2$, diluted sulphuric acid (0.1-0.5 M) and ammonia are commonly used in XOS production. High yield of XOS can be obtained by treating the substrates with dilute acid solutions at low temperatures for a limited period of time. A simple cost-effective pretreatment process of presoaking xylan rich substrates such as *Calamagrostis acutiflora, Miscanthus sinensis, Panicum virgatum* and bagasse in a dilute acid at lower temperature (60 ºC) for about 12 h followed by auto hydrolysis at high temperature (temperature ≥100 ºC for 1h) that yielded (65-92%) of XOS was also developed. On a commercial scale, this process is suitable for prebiotic production but the product contains monomeric sugar with contaminants such as furfural and HMF which are undesirable for food grade applications (Jain et al, 2015).

2.8.2 Auto hydrolysis: It is also known as hydrothermolysis, which is a non-chemical process that refers to deacetylation of D-xylan in the presence of water at higher temperatures. Due to the formation of acetic acid by partial cleavage of acetyl groups in the plant cell walls, auto hydrolysis takes place under slightly acidic conditions (pH ≤ 4.0). Concentration of XOS is dependent on the equilibrium between the breakdown of polymeric hemicelluloses to XOS and their further decomposition to monomeric xylose during auto hydrolysis treatment. Hence, XOS yield would be higher under moderate conditions. The treatments of increasing severity lead to decreased DP and increased decomposition of XOS into xylose. A researcher developed a process of XOS production from *Miscanthus giganteus*, which was subjected to auto hydrolysis at different temperatures (160-200 °C) for varied periods of time (15-60 min). At 160 °C for 60 min when the biomass was treated, highest yield of XOS was attained (about 65% of the dissolved xylan) (Jain et al, 2015).

A study used corn fiber separated from distillers dried grains as a feedstock for production of XOS and achieved 18.6 % substrate hydrolysis (Samala A et al, 2012). Another study treated bamboo culm with hot water in non-isothermal conditions (140-200^{o}C) for various time intervals (10-120 min) in a batch reactor which yielded a maximum of XOS (47.49 %) along with arabinose (0.20 g/L), galactose (0.19 g/L), HMF (0.15 g/L), furfural

(0.36 g/L), xylose (4.73%), acetic acid (0.64 g/L) and formic acid (0.26 g/L) (Xiao et al, 2013).

Due to re-polymerization of the lignin and xylan components, batch auto hydrolysis process leads to low hemicellulose sugar yields and hinders xylan hydrolysis into XOS which further complicates downstream purification (Jain et al, 2015).

2.8.3 Enzymatic

XOS can also be produced by enzymatic hydrolysis of xylan containing materials. Xylan-lignin complex is naturally resistant to enzyme attack; current commercial processes are usually carried out in a two-stage process: (1) alkaline extraction followed by (2) enzymatic hydrolysis (Qing et al, 2013).

Most of the plant materials contain xylan composed of β-1, 4-linked xylose units and various branching units including L-arabinose, D-glucuronic acid, 4-O- methyl glucuronic acid, $_{D}$-galacturonic acid, ferulic acid, coumaric, and acetic acid residues and to a lesser extent, $_{L}$-rhamnose, $_{L}$-fucose, and various O-methylated neutral sugars. Different enzymes are needed to work synergistically to completely hydrolyze these complex xylan structures. Generally, endo-β-1, 4-xylanases degrade xylan by attacking the β-1, 4-bonds between xylose units to produce XOS, and β-xylosidase converts lower-DP XOS into monomeric xylose. Enzyme mixtures with low endoxylanase and/or β-xylosidase activity are desirable in order to maximize production of XOS and minimize xylose production. In order to cleave xylan side groups, debranching enzymes such as α-L-arabinofuranosidase, *a*-glucu- ronidase and several esterases are needed. Fungi and bacteria that make multiple endoxylanase isoenzymes can also be produced *in situ*, reflecting the need for xylanases with specificities that are capable of acting on different substrates (Qing et al, 2013).

In contrast to chemical treatment methods and auto hydrolysis, enzymatic hydrolysis avoids production of undesirable by-products or high amounts of monosaccharides or high-pressure or high-temperature equipment. Enzymatic methods require much longer reaction times than acid hydrolysis or auto hydrolysis. Controlled production of XOS with a desired DP range can be more difficult because xylanase with different substrate specificities produces different hydrolysis end products. Acid hydrolysis of xylan

randomly hydrolyzes glycosidic bonds between adjacent xylose units (Qing et al, 2013).

Commercial xylanase preparations are often low in β-xylosidase activity, resulting in xylobiose accumulation (X_2) (Qing et al, 2011). Similarly, commercial cellulase preparations are usually low in β-xylosidase activity; that deficiency, coupled with the high inhibition of cellulase by xylooligosaccharides, has recently been shown to be an important contributor to reduced hydrolysis of xylooligosaccharides to xylose (Qing et al, 2011) as well as cellulose to glucose (Qing et al, 2010).

2.9 Purification of XOS

A variety of compounds such as monosaccharides, acetic acid, products derived from the extracted and acid soluble lignin fractions, soluble inorganic components of the feedstock, furfural from pentose dehydration and protein derived products appear in the reaction media are produced during the treatments of XOS production. Auto hydrolysis liquors need to be refined by removing both monosaccharide and non saccharide compounds to obtain a food grade XOS with a high XOS content. Usually, the purity of commercial XOS ranges between 75%-95% (Aachary et al, 2011). Purity and quality are two of the most important criteria to use XOS in functional foods (Qing et al, 2013).

2.9.1 Solvent extraction for purification of XOS

Solvent extraction is mainly used to remove the non-sugar components of hydrolysates (Aachary et al, 2011). The recovery and degree of purification of the XOS mixtures depend upon the solvent employed for extraction. Ethanol, acetone and isopropanol are the most common choices to refine crude XOS solutions (Jain et al, 2015).

2.9.2 Adsorption by Surface Active Materials

To separate oligosaccharides from monosaccharides or remove other undesired compounds, adsorption by surface active materials has been used in combination with other treatments. Most commonly used adsorbents for purification of XOS include activated charcoal, acid clay, bentonite, diatomaceous earth, aluminum hydroxide or oxide, titanium, silica, and porous synthetic materials (Qing et al, 2013).

A study used activated charcoal followed by elution with ethanol to fractionate XOs based on their molecular weight. In the first stage, XOs were retained by activated charcoal and then released according to DP by changing the ethanol concentration during elution (Qing et al,

2013). The highest XOs yield was achieved for elution with 15–30% ethanol, but only the total oligosaccharides concentration was measured by traditional post-hydrolysis with 4% sulfuric acid at 121°C for 1 hour and not the concentrations of each oligosaccharide DP fraction (Sluiter et al, 2010).

Another study proposed that treatment of raw XOS solutions obtained by auto hydrolysis of lignocellulosic materials by activated carbon is feasible for removal of extractives, lignin-derived compounds and carbohydrate degradation products. Selective adsorption of lignin products compared to carbohydrates was favored by three commercial activated carbons at slightly acidic pH. It also found that selectivity towards lignin adsorption was higher when the carbon used was highly microporous and had smaller mesopore diameters, a low volume of mesopores, and a low concentration of basic surface groups to favor adsorption of lignin derivatives (Montane et al, 2006).

2.9.3 Chromatographic separation for XOS purification

Chromatographic separation for XOS purification yields analytical grade high-purity fractions. Gel permeation chromatography (GPC), aqueous size exclusion chromatography (SEC), ion exchange chromatography (IEC) and centrifugal partition chromatography (CPC) are some of the commonly used techniques for purification of XOS (Jain et al, 2015).

A review article reported that hydrothermally treated LCMs have been fractioned by anion exchange chromatography and size exclusion chromatography. Chromatographic techniques have been used for refining samples before structural characterization of XOS. Simulated moving bed chromatographic separation has also been proposed for purification of oligosaccharides made up of xylose and arabinose units. Hemicellulose derived products were purified from hydrothermal microwave treatments of flax shive using ion exchange chromatography with enzymatic processing. Ion exchange was used for purification of XOS alone or in multi step processing, mainly for desalination and removal of other undesired by products or compounds. Most of the researchers were found using these purification techniques to obtain purified fractions of XOS for structural characterization. However, these methods are not cost effective and hence not feasible for large scale production of XOS (Aachary et al, 2011).

2.9.4 Membrane separation for XOS purification

Another powerful technology is membrane separation used in the purification of oligosaccharides. The most promising downstream processing strategy is ultrafiltration and nanofiltration based technique to manufacture high purity and concentrated oligosaccharides. It is a popular technique due to its low energy requirements, its relatively easy scale-up and easy to manipulate operational variables (Jain et al, 2015).

2.10 Sensory evaluation of food products

When the quality of a food product is assessed by means of human sensory organs, the evaluation is said to be sensory or subjective or organoleptic. Sensory quality is a combination of different senses of perception combing into play in choosing and eating food. The effective characteristic is not the property of the food, but the subject's reaction to the sensory qualities of foods. This reaction is highly conditioned by a variety of psychological and social factors and in the final analysis, plays a vital role in the acceptance and preference of foods (Srilakshmi, 2003).

2.10.1 Attributes of a food product

Various attributes of a food product are briefly described below:

2.10.1.1) Appearance: Appearance is the first characteristics perceived by the human senses and play an important role in the identification and final selection of food. This is the visual perception of food comprised of colour, shape, size, gloss, dullness and transparency. The appearances of a meal have shown impact on appetite stimulation or depression resulting in pleasure or total depression. The look of a food or beverage impacts craveability and acceptance, before the product touches the lips. This is because we eat with our eyes before we ever smell or taste (Sharif et al, 2017).

2.10.1.2) Flavour: It is sensory phenomenon which is used to denote the sensations of odour, taste and mouthfeel. Flavouring substances are aromatic compounds which are conceived by the combination of taste and odour and perceived by the mouth and nose. Odour improves the delight of eating e.g. aroma of freshly cooked rice and most of the baked products.Taste helps in identification, acceptance and appreciation of food.

It is perceived by the taste buds on the tongue. There are four types of taste perception: sweet, salty, sour and bitter. Sour and bitter are often confused. Lemon juice has a sour taste whereas coffee has a bitter taste. In case of mouthfeel, nerves present inside the mouth are enthused by chemical or thermal responses e.g. coldness of ice cream or the fiery impression of pepper (Sharif et al, 2017).

2.10.1.3) Aroma: Aroma is the first cousin of taste. These are volatile compounds which are perceived by the odour receptors of olfactory tissues of the nasal cavity. Aromatic compounds are released during the mastication process. Smell appraises the aroma of food that is important in the gratitude of flavour. A pleasant smell makes food delicious. To provoke a sensation of smell, the stuff must be in a gassy state. Furthermore, aroma is valuable in perceiving fresh, rancid or intermittently poisonous food (Sharif et al, 2017).

2.10.1.4) Texture: Texture is perceived by a combination of senses i.e. touch, mouthfeel, sight and hearing. It is one of the most imperative features of a food. If a customer bites a soggy biscuit or eats ice cream with sandy texture, it is improbable they will be back. Texture is prerequisite in the acceptance of numerous foodstuffs e.g. tenderness of meat and softness of bread. It also includes the consistency, thickness, fragility, chewiness and the size and shape of particles in food. Texture analyzer is helpful to guarantee the target texture from the laboratory to the user's kitchen (Sharif et al, 2017).

2.10.1.5) Sound: Hearing deliberates the sounds made by food during preparation and ingesting e.g. the crackle of fried food, the effervescence of drinks, the cracking of hard biscuits. So, in sensory analysis, the senses are used to measure, analyse and interpret the organoleptic or sensory properties of food (Sharif et al, 2017).

2.11 Types of tests

Different sensory tests are employed for food evaluation. The tests are grouped into four types.

a) **Difference tests:** a.1) Triangle test a.2) Paired comparison test a.3) Duo-trio test
b) **Rating tests:** b.1) Ranking test b.2) Single sample test b.3) Two sample difference test b.4) Multiple sample difference test b.5) Hedonic rating test b.6) Numerical scoring test b.7) Composite scoring test
c) **Sensitivity tests:** c.1) Sensitivity threshold test c.2) Dilution test
d) **Descriptive tests** (Srilakshmi, 2003)

a) Difference Tests: These tests are used in food industries to perceive minor differences in the samples but not the size of the difference. These tests can be accomplished by skilled as well as unskilled panelists. These are carried out to find out differences among the samples and how people notice and describe the difference. These are frequently used for screening and training of taste assessors. Difference testing is further classified into triangle test, paired comparison test, duo-trio test, multiple comparison test and ranking. The brief description of each is given below (Srilakshmi, 2003):

a.1) Triangle test: Triangle test can also be used for screening panelists who are able to perceive a difference. This test is valuable in quality control to detect ingredient substitution results and odd product from various manufacturing lots. These tests do not specify degree of amount of difference. For the purpose, the assessor should be requested to postulate dissimilar attribute. In triangle testing, each assessor gets three coded samples, one is different and two are identical. The task is to pick out the unusual sample. (Sharif et al, 2017).

a.2) Paired Comparison Test: This test is also recognized as the 2-AFC test (2 samples, alternate forced choice test). Each evaluator is provided with two coded samples and the task is to select the sample with the highest concentration of a pre-defined descriptor such as sweetness. This test is only meant for a detectable difference and does not specify the degree of difference. The likelihood of choosing the right sample by chance is 50%; hence, paired comparison test is more authoritative in finding differences than triangle test. A paired comparison

test is suitable for use in quality control; nevertheless, the exact characteristic evaluated is clearly stated must be known earlier (Sharif et al, 2017).

a.3) Duo-Trio Test: In this test, three samples are given to the judge; one is reference (labelled as R) and other two are coded. One coded sample is a duplicate of reference and other one is not similar. The assessor is asked to isolate the odd sample. It is mostly used with strong flavour products because less tasting is required. It is less effective than triangle test because the probability of selecting the correct answer by chance is 50%. It is less sensitive compared with triangle test as it is easier to conjecture the right one (Sharif et al, 2017).

b.1) Ranking: Ranking is a quick technique for evaluating numerous samples at once and is frequently used for screening of one or two of the best samples in a group. The assessor is provided with three or more coded samples and is asked to rank them for a specific trait. This test is similar to ranking for a primary taste but uses food samples rather than pure solutions (Sharif et al, 2017).

b.2) Single sample test: In this test, the panellist is asked to indicate the presence or absence and/or intensity of a particular quality characteristic. The completed analyses of two or more samples evaluated at different times can be compared with trained panellists. In market and consumer analysis, the results of different samples evaluated at different times by a different set untrained panellist can be compared (Srilakshmi, 2003).

b.3) Two sample difference test: This test is a variation of the paired test and measures the amount of difference. Each taster is provided four pairs of samples. Each pair consists of an identified reference and coded test sample. The test sample is a duplicate of the reference sample in two pairs. In other two pairs, the test sample is the test variable. The panellist is asked to judge each pair independently as to the degree of difference between the test sample and standard on a scale of '0' representing no difference to '3' representing extreme difference (Srilakshmi, 2003).

b.4) Multiple sample difference test: Each panellist is served with 3-6 samples. One sample is a known standard; panellist compares each coded sample with the

known standard. One coded sample is a duplicate of the sample. Direction and degree of difference is also to be judged (Srilakshmi, 2003).

b.5) Hedonic test: Hedonic rating depicts pleasurable or unpleasurable experiences. It asks each panellist to taste each sample and check a box from '1 Dislike very much to '5. Like very much' to indicate their preference. This is a 5-point scale and sometimes even a 9-point scale is also used (Srilakshmi, 2003).

b.6) Numerical scoring test: Each panellist is provided with one or more samples. The panellist evaluates each sample on a specific scale for particular characteristics indicating the rating of sample. The panellists are trained to follow the sensory characteristics corresponding to the agreed quality descriptions and scores (Srilakshmi, 2003).

b.7) Composite scoring test: In this test, specific characteristic of a product are rated separately. It is useful in grading products and comparison of quality attributes by indicating which characteristic is at fault in a poor product (Srilakshmi, 2003).

c. Sensitivity tests: Sensitivity tests are done to assess the ability of individual to detect different tastes, odour and feel the specific factors like hotness. These tests are used to select and train panel members for evaluating the quality of products containing spices, salt and sugar (Srilakshmi, 2003).

c.1) Sensitivity threshold test: It is defined as a statistically determined point on the stimulus scale at which a transition in a series of sensations or judgements occurs. There are mainly three types of threshold tests: Stimulus detection threshold, recognition identification threshold and terminal saturation threshold. These tests are also used where a minimum detectable difference of an additive or of an off flavour needs to be established (Srilakshmi, 2003).

c.2) Dilution test: This test is designated to establish the smallest amount of an unknown material developed as a substitute for a standard product. The quality of the test material is represented by the dilution number. The bigger the dilution numbers the better the quality of the test material (Srilakshmi, 2003).

d) Descriptive tests: This is a qualitative and quantitative description method for flavour analysis in product containing different tastes and odour (Srilakshmi, 2003).

Table 2.2: Number of Panellists and samples required for sensory test

Sl. No.	Method	Panellists		No. of sample tests
		Type	Number	
A.	**Difference**			
1.	***Paired comparison***	Trained Untrained	5-12 72-80	2
2.	***Duo-Trio***	Trained	5-12	3 (2 identical and 1 different)
3.	***Triangle***	Trained	5-12	3 (2 identical and 1 different)
B.	**Rating**			
1.	***Ranking***	Trained Semi-trained Untrained	5-12 10-15 72-80	2-7
2.	***Single sample***	Trained Untrained	6-25 72-80	1
3.	***Two sample difference***	Trained	6-25	4 pairs of unknown and control sample
4.	***Multiple sample difference***	Trained	6-25	3-6
5.	***Hedonic***	Semi-trained	10-25	5-10
6.	***Numerical scoring***	Untrained Trained	72-80 5-12	1-4 1-6 1-10
7.	***Composite***	Trained	5-12	1-4
C.	**Sensitivity**			
1.	***Threshold***	Untrained	-	5-10
2.	***Dilution***	Trained	12-24	5-10
D.	**Descriptive**			
	Flavour Profile	Trained	3-6	1-5

2.12 Selection and screening of Panel Members.

Assessors need to be screened by sensory expert regarding sensory perception. A variety of tests regarding the products under investigation and some general tasks required by the panellist are carried out by the sensory specialist. Screening test is suggested to be simple and not to over-test judges before performing true product assessments. A large number of screening tests could reduce the eagerness and motivation of the assessors at the time of actual evaluation. Medical screening is required before participation in the study, in certain situations (Sharif et al, 2017).

2.13 Training of panellists

In depth training is required for descriptive tests whereas only minimum training is prerequisite for discrimination tests which depend upon the level of sensory evaluation. The panellists must realize that sensory evaluation is a difficult task which requires full concentration and attention. Sometimes fresh assessors need to work with experienced judges who have been trained for other product categories for training purpose. The panellists get motivated and encouraged when they get appreciation from the top management (Sharif et al, 2017).

2.14 Technological benefits of adding prebiotics in foods

In food product development, sensory analysis is a decision making phase. Prebiotics added chocolate dairy dessert and sucrose replacement with different high-intensity sweeteners was developed. The analysis found that sweeteners had the highest sweetening power compared with the prebiotic chocolate dairy dessert containing 8% sucrose (Morais et al. 2014).

A study reported that addition of oligofructose in non flavoured yoghurt showed no influence on the pH, proteolysis or the viability of *Streptococcus thermophilus* or *Lactobacillus bulgaricus* during 28 days of refrigerated storage (Cruz et al. 2013).

Another study showed that inulin was supplemented in bread and resulted into smaller loaves, harder crumb and darker colour. It also reported that acceptability decreased with inulin content, inulin was degraded by yeast invertase and dry heat, and FOS or inulin fortification in bread at 5% looked achievable (Morris, 2012).

Effect of FOS on the sensory properties and consumer acceptability of peach-flavored drinkable yogurts showed non significant difference compared to the controls. This indicated that a prebiotic can be added without impacting acceptance (Gonzalez et al, 2011, Rolim, 2015).

FOS incorporated soup and beverages namely, butter milk, lemon juice, milk and tomato soup at 2.5%, 4%, 5%, 6% and 7.5% showed positive results on the

overall acceptability of the products (Gupta et al, 2011). A study fortified cookies and bread with prebiotic inulin (Parnami et al, 2010).

Another study developed a buttermilk based fermented drink using barley and fructooligosaccharide (FOS) as functional ingredients. A significant difference was reported among drinks with different flavors. Salt-jeera flavor was liked most by all the panellists followed by rose. No after taste or bad mouthfeel was reported in any of the products. The overall acceptability of the food products developed in the study was good due to the addition of FOS and due to its sweet taste it did not affect the taste, aftertaste and mouthfeel of the product (Hirdyani et al, 2016).

Different formulas of XOS 70P proved to have significantly higher consistency than 70L or 95P, based on the rheological measurement of aqueous solution. FOS, at low concentrations showed highest viscosity, while XOS 70L and 95P were the same as sucrose. Increase in concentration above 40(w/w) %, FOS showed lower viscosity than 70P due to changes of the hydration mechanism. Bigger differences were observed at a low temperature range (4–60°C) among viscosity of oligosaccharides than at higher temperatures (60–90°C). This indicated that refrigerated food products such as yoghurts, texture-modifying effect of XOS had primary importance. It was also reported that incorporation of XOS at low concentrations (1–3 w/w%) did not decrease gelatin gel strength but increased gelatin gel stability against mechanical with increasing amount of XOS. It concluded that texture modifying potential of XOS is different from other oligosaccharides (FOS or Suc) and hence needs to be considered during food product development (Penksza et al, 2019).

2.15 Future scope of prebiotics

A forecast has been reported that the global prebiotic market will exceed $8.5 billion by 2024. An estimate has also been made for probiotics that it will exceed $64 billion by 2022. The cost is borne by the consumer; costs vary tremendously between products and countries (Quigley, 2019).

Several in vivo and/or in vitro studies have been conducted to utilize probiotics and/or prebiotics. Various legal authorities worldwide has confirmed the safety of prebiotics and probiotics for food application, few studies reported incidences of bloating, flatulence, and high osmotic pressure, which can lead to gastrointestinal discomfort. Prebiotics and probiotics are believed to be safe for oral consumption because of their relatively low capacity to cause adverse effects (Yoo et al, 2016).

2.15.1 **Future scope of XOS as a prebiotic**

There is an emerging market for functional OS to be used in food. XOS is considered as potential ingredients in functional foods. For application of XOS as functional ingredients, a number of factors are responsible e.g. scientific research based evidence supporting that such foods help to improve and maintain overall health and well being, consumers demand, role of XOS for prevention of disease and self medication. XOS has advantages over other non digestible oligosaccharides in terms of both health and technological related properties (Gupta et al, 2016).

XOS has quite a number of health effects. XOS is recognized for interesting physicochemical properties; it has average sweetness, stable over a wide range of pH and temperatures and has sensory attributes suitable for incorporation into a variety of foods. It escapes from stomach's low pH and enzymes to colon and selectively stimulates the certain population of bacteria and acts as a non-digestible dietary component (Gupta et al, 2016). These properties of XOS make it a useful ingredient in novel functional foods.

Synbiotic is a synergy between probiotics and prebiotics which leads to development of foods containing both of them. An example of such a synbiotic food product is Bikkle, manufactured by Suntory Ltd. Japan since 1993. It is a drink comprised of *Bifidobacteria,* xylooligosaccharides, oolong tea extract and whey minerals with a pleasant odour and non-cariogenic characteristics (Kazuyoshi et al, 2016, Kazumitsu et al, 2016).

For special foods, XOS is a prospective food ingredient. It can be permitted in anti-obesity diets as it has low calories (Taeko et al, 2016). In food industry, XOS

has been found to produce low calorie sweeteners such as xylitol and antioxidant compounds. XOS has also been a flavour enhancer in formulating a beverage (Moure et al, 2006).

A positive impact on addition of XOS was found in a non-alcoholic carbonated drink with an intense sweetener (mixture of ace sulfame K and aspartame). It was reported that by adding the XOS, full bodied character of beverage was significantly enhanced without any drawback of off flavour perception or mouth-feel (Gupta et al, 2016).

2.15.2 Prebiotics in food industries

Incorporation of prebiotics in industrialized foods is now a healthy alternative, as people have become more health conscious and consumer demand for functional foods that can help in maintaining health has increased. The food industry is in an advantageous position due to the incorporation of prebiotics in food products as it improves the sensory characteristics, balances the nutritional composition in a better way and increases shelf-life (Al-Sheraji et al, 2013, Jailane et al, 2017).

Prebiotics are usually added to breakfast cereals, bakery products, beverages (e.g., fruit juices, coffee, cocoa, and tea), table spreads, butter-based products, desserts (ice cream, puddings, jellies, and chocolates) and dairy products (Al-Sheraji et al, 2013, Homayouni et al, 2014, Jailane et al, 2017).

A few studies also reported that prebiotics also have gelling properties which provide spreadable texture, maintain the emulsion stability, and water retention, thereby allows the development of processed foods with low fat content, with pleasant taste and texture (Al-Sheraji et al, 2013, Charalampopoulos et al, 2012, Jailane et al, 2017).

Some of the important characteristics of the manufacturing process, such as low pH, high temperatures, and conditions favoring the Maillard reaction must be considered while choosing the prebiotic to be incorporated to foods in order to

avoid anti-nutritional compounds formation which could be detrimental to the sensory quality of the final product and consumer health as well as the partial or total reduction of their action. XOS and GOS, among prebiotics has been found to be more stable at high temperatures and low pH mainly due to the beta bonds of their structure, which provide greater hydrolysis stability compared to FOS and inulin (Charalampopoulos et al, 2012, Jailane et al, 2017).

A study reported that RS3, a type of RS can also be added to fried battered products to increase dietary fibers content and avoid moisture reduction and fats absorption, since RS3 is very resistant to frying temperatures (Homayouni et al, 2014, Jailane et al, 2017).

Since prebiotics exert various technological benefits in food and many health benefits, food product development using prebiotics by the industry can be advantageous due to the demand and profitability of this market. Healthy foods will be available for consumers that can be readily consumed for the prevention or treatment of diseases, thus reducing public health costs. However, there is no consensus on the recommended quantity of specific prebiotics for consumption in the diet, and this limitation is a major challenge regarding the different in vitro and in vivo models used to test the prebiotic potential of foods (Jailane et al, 2017).

A study also mentioned that incorporation of prebiotics in meal replacement shakes and energy bars to increase the health component associated with them can be focused in future research. Prebiotic studies can be done in marine feed as a majority of population consumes aquatic foods. Food supplements and nutraceuticals developed from mushrooms to impart prebiotic effects and medicinal advantages such as anti-tumor effects and immune modulation require extensive studies. In this area of functional foods, more researches are coming up and hence, new prospects are opened for the food industry to raise the health component of their products (Singla et al, 2017).

Further research is required to reduce the cost of production and clinical trials for safe human consumption and to elucidate their role in the reduction of cancer risks as well as in getting relief from other ailments. There is a scope to produce XOS by selecting different substrates, enzymes, LCM pre treatments and xylan treatments for improving the yield and quality. From the literature review, it is found that the pre-treatment of LCMs with alkali is likely to be the most efficient and safest (Jain et al, 2015).