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Production, Properties and Applications of Xylooligosaccharides (XOS): A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Xylooligosaccharides derived from Xylan, significant component of hemicellulose in lignocellulosic plant biomass, are essential raw materials used in the food, pharmaceutical and agricultural industries. They find application in these industries due to their unique physicochemical properties such as pH stability, growth regulatory ability, and anti-allergic property. In addition, they have been shown to exhibit promising antimicrobial, anti-cancer, antioxidant and anti-inflammatory activities. Xylooligosaccharides convert waste into valuable foods, pharmaceuticals, and agricultural products, promoting health, the economy, and the environment.

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1. INTRODUCTION

Carbon availability in lignocellulosic resources (trees, grasses, agricultural residues) promotes its applicability in energy and materials sectors, mainly as fuel and building materials [1]. The average lignocellulosic plant biomass contains 40 –50% cellulose, 15 -25% lignin, and 23 –30 % hemicellulose. Hemicelluloses are large groups of homogenous and heterogeneous polymers of pentose namely xylose and arabinose; hexose (mannose, glucose, and galactose); and sugar acid (containing two to six of these sugars), which connects the cellulose and lignin (in plants structures), tightly interconnected within the cell wall [2]. Their composition and percentage in plant species depend on age, growth stage, and conditions under which the plant develops [3]. Hemicellulose is classed based on the primary sugar residues in the backbone units notably xylopyranose, glucopyranose, mannopyranose, and galactopyranose. These backbones are linked to different side chains and can be hydrolyzed to monosaccharides. The number of side chains and side chain residues composition varies depending on the kind of biomass, cell wall types, and life stages of the plant [4]. Many researchers have recently studied their chemical, pharmaceutical, and biotechnological values (Eero, 2015).

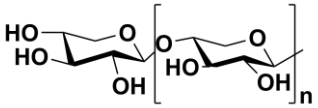
Xylan is a heteropolysaccharide that is the primary component of hemicellulose. They are non-starch polysaccharides that share a β-(1-4)-linked xylopyranose mainly substituted with L-arabinose furanose or its feruloylated derivatives at 0-2 or 0-3 positions. Xylan comprises 15 - 30 % hardwoods and roughly 7 – 12 % softwood species. Despite its abundance, the suitability of Xylan as a specialty biopolymer is limited in comparison with cellulose due to a higher degree of substitution and lower degree of polymerization, causing it to be soluble upon dispersion in water [4,5,2,6].

Xylan is the principal source of various commercially valuable products such as xylose, xylitol, and xylooligosaccharides. These products can be used in industrial operations to manufacture valuable products such as sweeteners. In this study, we will discuss the methods of extracting, and purifying, and the recent applications of xylooligosaccharides in numerous fields.

2. XYLOOLIGOSACCHARIDES (XOS)

Xylooligosaccharides (XOS) are a family of non-digestible dietary components made up of D-xylose units linked by β-1,4 – glycosidic bonds formed by the hydrolysis of Xylan. They are polymers of the sugar-xylose consisting of xylobiose, xylotriose, and xylotetrose [7-9]. XOS occurs naturally in bamboo shoots, fruits, vegetables, milk, and honey [10] and can be produced at a large scale from xylan-rich materials such as forestall, agricultural or industrial waste of lignocellulosic nature. Xylooligosaccharides are favorable over other non-digestible oligosaccharides, such as fructo-oligosaccharides, in terms of medical and technological-related properties. According to Gupta et al. [11], XOS have been reported to be extracted from several plants such as rice bran, wheat, *Eucalyptus pellita*, and finger millet [12,13]. XOS are stable over a broad range of pH and temperatures. They exhibit a range of health benefits and beneficial changes to immune makers. However, the growing commercial relevance of these non-digestible oligosaccharides depends on their beneficial health qualities, notably the prebiotic activity [7,13,11].

The structures of xylooligosaccharides vary in degree of polymerization, monomeric units, and types of linkages based on the xylan sources utilized for production. These xylan sources include corn cobs, wheat straws, tobacco stalks, sugarcane bagasse, rice hulls, malt cakes, bran, and cotton stalks [14].



Where **n** is a variable number of xylose units

Fig. 1. Basic structures of xylooligosaccharides

3. PROPERTIES OF XYLOOLIGOS-ACCHARIDES

The properties of XOS can be grouped into physicochemical and biological properties.

3.1 Physicochemical Properties

Xylooligosaccharides are water soluble and highly hygroscopic. They are particularly stable in acidic media (pH 2.5 – 8.0). They are non–toxic, less sweet, and can be used as reinforcing agents in drinks. XOS contain low caloric values and appropriate organoleptic characteristics (i.e. mouth feel, taste, texture, and colour) suited for incorporation into food. They are resistant to heat (above 100 ^{0}C) and can be employed as stabilizing agents [11]. With the presence of β-bonds and their acid stability properties, XOS are protected from degradation through the stomach [15].

3.2 Biological Properties

Xylooligosaccharides are non-digestible. They exhibit bacteriostatic actions and favour the selective proliferation of Bifidobacterium. XOS also demonstrate potential as enzyme inhibitors and anti-cancerous agents [7, 9].

4. PRODUCTION OF XYLOOLIGOSACCHARIDES

Xylan-rich lignocellulosic materials can be effectively used to produce xylooligomers through fractionation processes. These fractionation processes involve

- Selection and pretreatment of Lignocellulosic materials,
- Isolation or extraction of precursors (xylan),
- Breaking down of precursors into XOS using chemicals or enzymes,
- Purification to obtain high-quality XOS.

4.1 Selection and Pretreatment of Lignocellulosic Materials

The extraction of Xylan from lignocellulosic materials is often harrowing because of the influence of the side chain substitution pattern, physical entanglement, and covalent bonding between the carboxyl group of uronic acid and the hydroxyl group of the Xylan backbone [2]. The purpose of pretreatment is to disrupt the plant cell wall structure to make hemicelluloses more accessible and enhance the effective yield of Xylan by weakening the cellulose-lignin network. The pretreatment process can be selected based on the type of lignocellulosic biomass and process cost. However, it must involve the following:

- Physical treatment involves increasing the surface area of the biomass (i.e. particle size reduction by grinding or milling). This leads to structural changes and chemical bonding distortion due to imposed stress.
- Chemical treatment using acid, alkali, peroxide or enzymes.
- Delignification using oxidative agents. This involves the liberation of lignin in the biomass [2].

4.2 Extraction of Xylan

Xylan extraction from the cell wall is based on solubility. The procedures include water extraction and alkali extraction. The ratio of sugar units in Xylan depends highly on the extraction procedures. The acetyl groups are typically left on the Xylan unsubstituted to allow them to maintain their water solubility. Water extraction facilitates the isolation of high molar mass xylan with minimal arabinose substitution. This helps retain the Xylan structure, although the yield will be low [16,2,17]. Sodium hydroxide, potassium hydroxide, and barium hydroxide are typical alkalis utilized in alkali extraction at high temperatures (90°C). The alkali extraction is disadvantageous because it leads to deacetylation, whereby the original structures are not retained resulting in shrinkage. Thus, water-based extraction is helpful for the preservation of the xylan structure.

4.3 Production of Xylooligosaccharides from Xylan

The production of xylooligosaccharides from Xylan comprises two processes: thermochemical production, and enzymatic hydrolysis. It was observed that enzymatic procedures are preferred in the food or pharmaceutical sectors to avoid forming degraded products. At the same time, chemical methods are recommended to produce XOS mixtures with a wide degree of polymerization range [18]. In the chemical production of XOS, dilute mineral acids like H_2SO_4 and strong alkalis like KOH and NaOH are employed. The degree of polymerization of the XOS produced using the acids and alkali depends on the concentration of the reagents, temperature, and reaction time [15,18].

4.4 Thermochemical Production of XOS

“XOS commonly use steam and dilutes mineral acids or alkaline solutions. The single-step

production of XOS by reaction with moisture or water by hydronium-catalyzed degradation of Xylan is known as autohydrolysis, hydro thermolysis, or water hydrolysis. Autohydrolysis occurs at slightly acidic conditions (pH < 4) generated by the acetic acid released by partial cleavage of acetyl groups on the Xylan. In the initial stage of autohydrolysis, hydronium ions are formed through the auto-ionization of water under high temperature and pressure" [16]. As the reaction proceeds, cleavages of acetyl groups from the xylan backbone form acetic acid and contribute more hydronium ions. Adding more acids beyond those released naturally from the biomass may enhance Xylan breakdown into XOS. Also, [19] were reported to have produced XOS in good yield by autohydrolysis of corncob biomass at 150°C [15]. Another team of researchers also synthesized XOS via hydrothermal processing of wood chips of *Eucalyptus nitens*. The synthesis was done by heating a mixture of *Eucalyptus nitens* wood chips and distilled water in a reactor at a liquid-to-solid ratio (LSR) of 8 kg/kg [oven-dry wood basis], up to a temperature of 195°C. Autohydrolysis products were obtained after cooling and centrifugation. Mixtures of substituted XOS obtained were refined through a two-step membrane filtration and ion exchange [20].

"Autohydrolysis has the advantage of removing harsh chemicals from the extraction and hydrolysis of Xylan but requires equipment that operates at high temperatures and pressures. The molecular weight distribution of XOS generated by autohydrolysis after purification comprises a considerable percentage of a high degree of polymerization compounds (MW 1000 – 3000g/mol) and a small fraction of low degree of polymerization compounds (MW < 300g/mol)" [16,2].

4.5 Production of XOS by Enzymatic Hydrolysis

"This technique is known as Green technology. This includes the enzymatic conversion of Xylan into xylooligosaccharide by the actions of endo-xylanase. The debranching enzymes such as α – L – arabinofuranosidase, α – glucuronidase, and several esterases first cleave the xylan side group" [21]. "The endo – β – 1, 4 – Xylanase then degrades Xylan by attacking the β – 1, 4 – bonds between xylose units to produce XOS. β – xylodase converts lower DP XOS into monomeric xylose. To optimize the production of XOS and reduce xylose production, enzyme mixtures with low endo-xylanase and β – xylosidase are employed" [2,8].

"In another study, a cost-effective approach to enhancing growth probiotics using XOS syrup produced using agricultural lignocellulosic waste, wheat bran, as substrate" was also shown by [22]. "They discovered that among tested probiotic strains, Lactobacillus Brevis displayed maximum growth in the presence of 0.5% XOS syrup with a specific growth rate of 1.2/h. Similarly, XOS syrup has also been proven to have been produced from almond-shell hemicellulose consisting primarily of xylobiose and xylotriose. On in vitro fermentation of the concentrated XOS utilizing different strains of *Lactobacillus* and *Bifidobacterium*, *L. acidophilus*, *B. adolescentis*, and *B. breve* were shown to have fermented XOS to a varying extent, as indicated by their difference in growth, to produce acetate as a predominant short-chain fatty acid" [23]. Meanwhile, XOS were reportedly made from water-soluble Xylan which was synthesized from a finger millet seed coat. When treated with xylanase of *Thermomyces lanuginosus*, water-soluble Xylanase generated 75% xylooligosaccharides. The XOS were stated to have powerful antioxidant and prebiotic efficacy, and significant antibacterial activity compared with commercially available XOS [24]. Also, "white-rot fungus *basidiomycetes* Pleurotus sp. BCCB068 and *Pleurotus tailandia* were used to degrade oat-spelt Xylan to XOS. It was discovered that endo-1,4-β-xylanase and β-xylosidas were active" [25].

Enzymatic hydrolysis, as opposed to thermochemical or autohydrolysis, eliminates the production of unwanted by-products or a large number of monosaccharides with no control over the degree of polymerization. It also does not need robust equipment operating at high temperatures and pressures. It is also resistant to alkalis and acids [15,18,12,21]. "Enzymatic hydrolysis, on the other hand, takes longer reaction durations and is more costly than autohydrolysis. Furthermore, Xylanase with different substrate specificities produces different hydrolysis end-products, making it more difficult to control the production of XOS with the desired degree of polymerization range. Autohydrolysis is suitable for creating XOS with degrees of polymerization ranging from 2 to 15" [8,12].

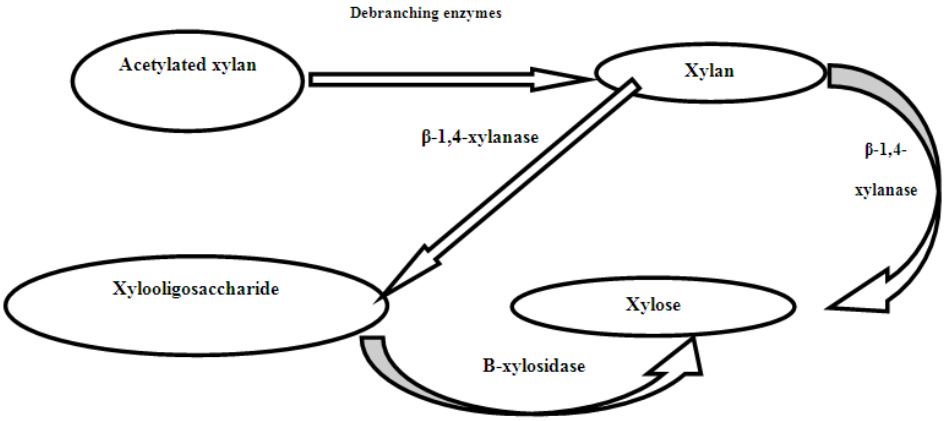


Fig. 2. Enzymatic hydrolysis of xylan to XOS and xylose

5. XYLOOLIGOSACCHARIDE SEPARATION AND PURIFICATION

XOS derived from thermochemical or enzymatic treatment usually contain a wide degree of polymerization (DP) range of oligomers, and purification of the XOS to obtain XOS with a desirable range of DP has proven to be a significant challenge, especially for XOS derived from the hydrothermal process [26]. "To produce xylooligosaccharides fractions used in the food and pharmaceutical industries, the XOS must be refined by eliminating monosaccharide or non-saccharide compounds to create the highest possible xylooligomer content" [8,2]. Purifying and separating XOS from autohydrolysis liquor is complex and may require many reactions and fractionation stages. Depending on the level of purity sought, a series of physiochemical treatments may be necessary. Organic acids including ethanoic, methanoic, and propanoic acids have been reported to be employed in the extraction of XOS from Xylan, whereas propane-2-one and alkanols have been recommended for XOS recovery [26]. To overcome the difficulties associated with chemical and enzymatic hydrolyses, Jang et al. [12] used hydrothermal treatment (liquid hot-water treatment) to extract XOS from *Eucalyptus pellita*. They reported that the xylan extraction ratio based on the initial xylan content of the feedstock was maximized up to 77.6% at 170^0C for 50 min condition, accounting for XOS purity of 76.5% based on the total sugar content of the liquid hydrolysate. The sum of xylobiose, xylotriose, and xylotetraose with a low degree of polymerization (DP) of 2 to 4 was found to be 80.6% of the total XOS in this condition. In addition, Miguez et al. (2021) got 84% substituted XOS in their study.

5.1 Solvent Extraction

XOS are commonly recovered by solvent extraction. To ease purification, it is sometimes used to pre-extract interfering components before thermochemical or enzymatic treatment. Non-saccharide compounds can be removed by solvent extraction, producing both a refined aqueous phase and extractive-derived compounds. The solvent used for extraction determines the degree of purification and the yields recovered. The most common solvents used to refine crude XOS solution are ethanol, acetone, and 2-propanol, with ethanol producing the maximum purity at the expense of lower recovery yields [2]. Due to their comparable water solubility and molecular weights, XOS and soluble lignin are difficult to separate in prehydrolyzate. In their research, Huang et al. [27] isolated soluble lignin and XOS from pre-hydrolyzate derived from the pre-hydrolysis process in the dissolving pulps industry. In addition to producing high-quality XOS, the group observed that there is stimulation of the production of short-chain fatty acids by *Lactobacillus acidophilus* as well as antioxidant activity of XOS and XOS promoting the growth of intestinal *Bifidobacterium adolescentis*.

5.2 Adsorption by Surface-active Materials

Adsorption by surface active materials has been combined with other treatment steps to separate XOS from monosaccharides and other undesired compounds. The most widely used adsorbents for the purification of XOS include acid clay, diatomaceous earth, activated charcoal, bentonite, aluminum hydroxide, titanium, silica,

and porous synthetic materials. Activated charcoal followed by elution with ethanol in fractionating XOS requires the retainment of XOS by activated charcoal in the first stage. The XOS are then released according to the degree of polymerization by changing the ethanol concentration during elution. This method is feasible for removing extractives, lignin-derived compounds, and carbohydrate degradation products [2,26].

5.3 Chromatographic Separation Technique

Unlike solvent extraction and adsorption by surface active material, which does not ensure adequate XOS purity, the chromatographic separation techniques yield the purest XOS fraction. Size-exclusion chromatography (SEC), ion-exchange chromatography, charcoal chromatography, and gel permeation chromatography are all examples of the chromatographic technique. Because of its higher loading capacity than other separation techniques, charcoal chromatography is often preferred for sugar purification. However, utilizing this approach to separate XOS with a high degree of polymerization is challenging, because XOS with uronic acid substituents combine with simpler XOS on the chromatograph. As a result, a gel permeation chromatograph containing cross-linked polyacrylamide and dextran beads is used [2,26]. Reddy and Krishnan [28] isolated xylobiose produced by crude enzymes from *B. subtilis* using high-performance liquid chromatography (HPLC) [15].

5.4 Membrane Filtration Technique

This technology is made up of nanofiltration and ultrafiltration. While nanofiltration concentrates XOS by removing low molecular weight substances like phenolic compounds and monosaccharides, ultrafiltration separates XOS from high molecular weight compounds with varying degrees of polymerization (DP) [29,19]. Yuan et al. [30] and Geetha and Gunasekaran, [22] employed nanofiltration and ultrafiltration techniques to recover 44.4% and 74.4% of XOS produced by *Bacillus sp*., respectively [15].

Membranes have many benefits over the other purification methods mentioned above, including low energy requirements, effective control of critical operating parameters, and relatively simple scaling up [31]. However, some oligosaccharide structural features, such as the type of monosaccharide, links, and substitutions of the oligomer structure, and oligosaccharide solubility, could influence the membrane filtering process [32].

6. APPLICATIONS OF XYLOOLIGOSACCHARIDES

6.1 Food Industry

XOS have shown several practical, including medicines, feed formulations, culinary and agricultural applications. Their most major market development, however, relates to food-related applications. For food-related applications, XOS with a polymerization degree of 2 - 4 is preferred [17]. As a result, the production of XOS from an efficient and cost-effective xylanase-based bioprocess or autohydrolysis process is needed. Beyond adequate nutrition, food is considered functional if it influences one or more target bodily functions that enhance health and well-being or decreases the risk of illness. XOS are regarded as non-digestible food ingredients and potential functional food ingredients. They are frequently used in the food industry as prebiotics and additives [33,11].

6.2 Prebiotics

Prebiotics are essential for improving human gastrointestinal health and have been linked to decreased symptoms of irritable bowel syndrome, better digestion and gut function, immunological regulation, potential protection against colon cancer, and possibly management of obesity [10]. Prebiotics are fermented nutrients that permit specific changes in composition and activity in the gastrointestinal microbiota, conferring benefits on host well-being and health [11]. Baker et al. [6] reported that using XOS had physiological impacts on intestinal health and growth performance in monogastric animals. To be considered a prebiotic, an oligosaccharide must not be hydrolyzed or absorbed in the upper part of the gastrointestinal tract. It must be selectively assimilated by one or a limited number of helpful microorganisms in the colon, boosting either luminal or systemic effects [17]. XOS are neither digested nor absorbed (and hence could function as prebiotics); alternatively, they reach the colon immediately and are preferably utilized by Bifidobacterium to multiply beneficial bacteria for the human body, thus maintaining a favorable intestinal environment. In addition, XOS inhibit the growth and proliferation of other harmful bacteria in the intestines. XOS

have a 20-fold greater effect on Bifidobacterium than other polymer sugars such as fructooligosaccharide (FOS) [7]. XOS have a good impact on bowel function as well. Because they are not easily digested by digestive enzymes, an overdose would result in diarrhea due to significant moisture retention. This form of gastrointestinal pain is commonly associated with a high fibre intake and is not harmful to individuals [21].

Furthermore, Yang et al. [34] in their work investigated "the effect of the prebiotic xylooligosaccharide on the gut microbiota in both healthy and prediabetic (Pre-DM) subjects, as well as impaired glucose tolerance. They revealed that prebiotic XOS may be beneficial in reversing changes in the gut microbiota during the development of diabetes". XOS supplementation may help manage obesity since it did not increase *Lactobacillus* (a member of the Firmicutes). Obesity-associated gut microbiota has been reported to have increased numbers of *Lactobacillus* and an increased Firmicutes/ Bacteroidetes ratio [10].

In another work, Lin et al. [35] studied "the prebiotic effects of XOS on the improvement of microbiota balance in human subjects. They reported that the intestinal microbiota balance was improved after daily consumption of 150g of rice porridge containing XOS for 6 weeks, demonstrating the prebiotic potential of XOS incorporated into foods".

6.3 Food Additives

Besides their health benefits, XOS have intriguing physicochemical properties; they are moderately sweet and have a pleasant odor. They are stable over a wide temperature (up to 1000°C) and pH range (2.5 – 8.0). They are non-carcinogenic and have low-calorie content [17]. These qualities make them excellent for incorporation into a wide range of food products, including soymilk, soft drinks, dairy products, sweets, and confectionaries.

XOS are potential food ingredients for high-end foods. They are low in calories and could be employed in anti-obesity diets (Izumi et al. 2009). XOS outperform other oligosaccharides and insulin in carbonated beverages, low-pH juices, and acidic foods. XOS can equally be utilized in producing low-calorie sweeteners such as xylitol and anti-oxidant compounds. They may also be employed as flavour enhancers in beverage formulation. The addition of XOS to non-alcoholic carbonated beverages with intense sweetness has been demonstrated to have a positive impact [2,9]. The rate of XOS incorporation has a significant effect on moisture content, pH, and acidity. Adding XOS up to 3.5% does not affect the taste and overall acceptability, but the aftertaste does [9].

7. OTHER APPLICATIONS OF XYLOOLIGOSACCHARIDES

"XOS have found their use in pharmaceuticals such as antiviral and anticancer medications. In an *in vitro* study, it was discovered that a fraction of xylose, XOS, and water-soluble lignin has cytotoxic effects and reduces the viability of acute lymphoblastic leukemia cell lines" [11]. They have also exhibited immunomodulatory, anti-cancer, anti-microbial, growth regulator activities, and other biological activities such as anti-oxidant, anti-allergic, anti-inflammatory, and antihyperlipidemic, among other things. XOS have been proven in research to reduce pancreatic insulin release, increasing intestinal mineral absorption. XOS consumption effectively reduces severe constipation in pregnant women with no adverse side effects [2]. In addition, "XOS have shown to modify the intestinal mucosal barrier and the cecal microbiota in laying hens fed with oxidized fish oil. They were reported to have reversed the effects of oxidized fish oil on hen production performance, ileal mucosal secretory immunoglobulin A content, increased serum endotoxin concentration, and claudin-1 and claudin-5 mRNA expression in the ileal mucosa. The XOS corrected these changes and enhanced the villus height-to-crypt depth ratio at 400 mg/kg. Supplemental XOS also altered the cecal microbiota of layers fed the oxidized fish oil diet, resulting in an increase in microbial richness and changes in microbial composition, including increases in *Firmicutes*, *Ruminococcaceae*, *Verrucomicrobia* (*Akkermansia*), *Paraprevotella*, *Prevotella*, and *Oscillospira*, as well as a decrease in Erysipelatoclostridium spp" [36].

"Furthermore, XOS were reported to have shown immunomodulatory effects. In a concentration-dependent way, XOS in the *in-vitro* model inhibited TNF-α, IL-1β, IL-6, and Nitric oxide production and induced IL-10 production in lipopolysaccharide (LPS)-stimulated RAW264.7 cells without imposing cytotoxicity.

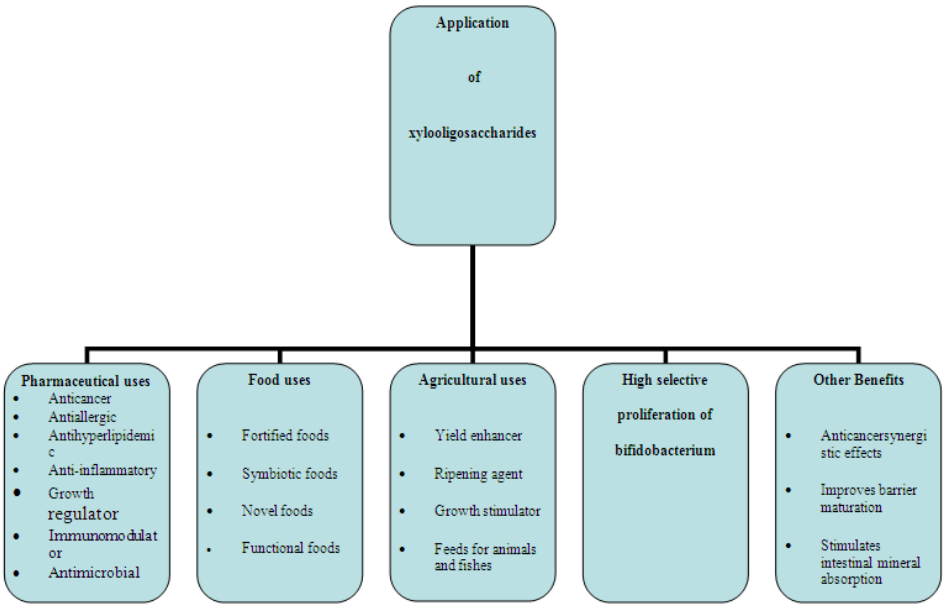


Fig. 3. Application of xylooligosaccharides

It was also discovered that prostaglandin E2 was not altered throughout the treatment" [14]. Meanwhile, in their study, Hsu et al. [37] evaluated "the effects of XOS on the alteration of cecal microbiota, cecal pH, cecal weight, and serum lipid levels, and their inhibitory effect on pre-cancerous colon lesions in male Sprague-Dawley rats. The rats were fed with 1,2-dimethylhydrazine (DMH) [15] mg/(kg body wt/wk) for 2 weeks and also treated with DMH +60 g XOS/kg diet, it was observed that XOS dramatically lowered the cecal pH and serum triglyceride concentration, and increased the total cecal weight and bifidobacterial population. Moreover, XOS dramatically decreased the aberrant crypt foci in the colon of DMH-treated rats".

8. CONCLUSION

Xylooligosaccharide-derived lignocellulosic plant biomasses have shown tremendous potential in industrial applications. XOS may be added to various food products owing to its functional properties and benefits. XOS have significant potential as prebiotics to maintain and promote a balanced microflora for improved health and well-being, which might lead to their use in cancer and other treatment procedures. However, the mechanisms involved in these processes will need to be investigated further in both bench and clinical studies.

There is currently a scarcity of data on XOS concerning prebiotics. The information presently available does not give a precise explanation of XOS's effects. More study on XOS is therefore urged. Furthermore, XOS have shown significant potentials in food, and other industries including pharmaceutical, agriculture, and deed formation. More research in these areas is equally suggested.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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