

## Valorization of Agricultural Waste into Xylooligosaccharides: A Sustainable Source of Functional Ingredients

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Xylooligosaccharides (XOS) are non-digestible oligosaccharides made primarily of xylose units with a high prebiotic potential. XOS display great thermostability (100 °C) and strong acid resistance over a pH range (2–7), making them suitable for gastrointestinal administration given their potential to tolerate gastric acid. XOS improves digestion and nutrient absorption while also providing health advantages by inhibiting the formation of harmful microorganisms. XOS, generated from lignocellulosic biomass (LCB) through enzymatic or chemical hydrolysis, have significant prebiotic characteristics and broad applications in medicine, food, and health. The XOS purification has become crucial to their subsequent industrial application. Still, it needs further exploration to lower costs and increase the purity of the XOS-supplemented diet. It benefits host wellness in numerous ways because of its immunomodulatory, growth-regulating, anticancer, antibacterial, antioxidant, and other bioactive compounds. Lignocellulosic wastes have the ability to be transformed into oligosaccharides and other value-added products. The synthesis of oligosaccharides from renewable sources bestow to growing bioeconomies. Oligosaccharides are synthesized chemically or biologically from agricultural residues. These oligosaccharides are functional food supplements that have a positive impact on humans and livestock. Non-digestible oligosaccharides, referred as prebiotics are beneficial for the colonic microbiota inhabiting the digestive system. These microbiota plays a crucial role in stimulating the host immune system and other physiological responses. The commonly known prebiotics, galactooligosaccharides (GOS), xylooligosaccharides (XOS), fructooligosaccharides (FOS), mannanooligosaccharides (MOS), and isomaltooligosaccharides (IOS) are synthesized either through enzymatic or whole cell-mediated approaches using natural or agricultural waste substrates.

**Keywords:** Xylooligosaccharides; XOS; agricultural by-products; lignocellulosic biomass; prebiotics; enzymatic hydrolysis; biorefinery; functional ingredients and circular bio-economy.

### Introduction

Active research on converting agricultural by-products into value-added products has been purposed to produce different biomolecules of great economic value; prebiotic xylooligosaccharides (XOS) is one of them (Gullon et al., 2010). The depolymerized products of xylan such as xylose and XOS have potential applications in food, pharmaceuticals, feed formulations, and agricultural purposes. XOS is a non-digestible food ingredient, made up of d-xylose units linked by  $\beta$ -1, 4 glycosidic bonds, has a lower degree of polymerization (DP 2–10) (Jaypal et al., 2013). XOS production by autohydrolysis or hydrothermal processing of lignocellulosic biomass results in hydrolysates containing a large variety of undesirable components, such as xylose and lignin, demanding a complex purification step (Zhu, Kim, Lee, Chen, & Elander, 2006). Thus enzymatic hydrolysis is generally preferred by food industries due to it does not produce undesirable by-products neither requires special equipment operating

at high-temperature and high-pressure conditions, in contrast to autohydrolysis (Akpınar, Erdogan, & Bostancı, 2009). XOS can be produced by endoxylanases, which hydrolyze the  $\beta$ -1,4-glycosidic linkages in xylan. Actually knowledge about the xylanolytic activity, optimum pH and temperature, thermal stability, and kinetic parameters of a commercial xylanase preparation is pivotal to achieve relevant XOS yield as well as feasible large-scale production. Comprehensive studies of the mode of operation ratio of enzymes in cocktails can lead to positive results for rational designing of enzymatic mixtures with better efficiencies which are also cost effective promoting synergistic effect on hemicellulose conversion (Goldbeck et al., 2014; Ávila, Cairo, Damasio, Forte, & Goldbeck, 2020). Furthermore it is known that complex substrates provide from lignocellulose materials contain large amounts of arabinoxylan and lignin which are not easily degraded by endo-xylanases without prior or simultaneous treatment with arabinofuranosidases and feruloyl esterases (Ávila et al., 2020).

XOS are used in food industries and are incorporated into many food products with different applications such as sweeteners, stabilizers, emulsificants and functional ingredients (Gullon et al., 2010). One of the most important applications of XOS is as functional foods, since they are considered prebiotic compounds, due to capacity to promote beneficial health effects for their selective metabolism by bifidobacterias and lactobacillus, increasing the production of volatile fatty acids, and anti-gastric ulcer activity (Vazquez, Alonso, Dominguez, & Parajo, 2000).

### **The Importance of Xylooligosaccharides as Prebiotics**

Xylooligosaccharides (XOS) have great prebiotic potential and can be incorporated into many food products. XOS seem to exert their nutritional benefits in various animal species, which by definition have an intestinal tract populated by a complex, bacterial intestinal ecosystem. The production of XOS on an industrial scale is carried out from lignocellulosic materials (LCMs) rich in xylan by chemical and enzymatic methods and the latter is preferred in the food industry because of the lack of undesirable side reactions and products. The information available does not provide an exact explanation about the bioactive effects of XOS. Their fermentation results in the acidification of the colonic contents and the formation of short chain fatty acids that serve as fuels in different tissues and may play a role in the regulation of cellular processes.

### **Agro-Industrial by-Products as Substrates for Xylooligosaccharides Production**

The cost of the production of XOs is a limiting factor, so their obtention from agro-industrial waste, including many grain by-products, has been considered a potential cost-reduction strategy as this is a low-cost, renewable, and abundant raw material. Despite the benefits of using agro-industrial residues, their varying composition is a challenge, because depending on the chemical composition, the residues may be more or less suitable for XOs production. Residues with higher amounts of xylan and low amounts of lignin are better options. Corn cob, wheat straw, rice straw, corn stover, switchgrass, and sugarcane bagasse are some of the relevant residues for XOs production, due to their high levels of hemicellulose. Xylan, the second most abundant biopolymer in nature, constitutes most of the hemicellulose that can be degraded into XOS, using combinations of pretreatments and enzymatic hydrolysis. The high xylan content of corn cob is considered attractive for XOS production.

#### **1. Lignocellulosic Biomass Pretreatment**

Agro-industrial by-products are mostly composed of cellulose, hemicellulose, and lignin structures. Cellulose and hemicellulose can be enzymatically hydrolyzed into glucose and xylose; however, cellulose is strongly associated with hemicelluloses and lignin, preventing the access of hydrolytic agents, and its crystalline structure is also an extra obstacle to hydrolysis. To increase the efficiency of the use of lignocellulosic residues, it is necessary to perform a step before the enzymatic hydrolysis, called pretreatment, to extract xylan from the plant cell wall. Several pretreatment techniques have been reported in the literature, such as steam explosion, solvent extraction, and thermal pretreatment using acids or bases, organosolv, deep eutectic solvents, and hydrothermal pretreatment. During pretreatment, hemicellulose

can be depolymerized to produce various xylosugars, among them short-chain polysaccharides with different degrees of polymerization, which are considered attractive because of their potential use to promote the growth of intestinal bacteria and improve immunity

## **2. Enzymatic Hydrolysis for Xylooligosaccharides Production**

Enzymatic methods are commonly utilized to obtain XOs, especially due to their high yields and specificity in generating products. The advantages also include the use of milder operating conditions, resulting in less generation of polluting waste. The enzymatic method can be used either with enzymes in biological treatments or applied after physicochemical pretreatment to increase XOs production from lignocellulosic biomass. The group of enzymes involved in this method is called hemicellulases, which can degrade hemicellulose into smaller polymers and sugars. Xylanases (endo-1,4- $\beta$ -xylanases, EC 3.2.1.8) are one of the most representative enzymes within this group, classified as endo enzymes that catalyze the hydrolysis of the  $\beta$ -1,4 linked chains of xylose to produce small XOS. Xylanases are grouped as glycoside hydrolases (GH) in the families of glycosidases 10 (GH10) and 11 (GH11) [72]. GH10 enzymes have a preference for groups at the terminal of xylan bonds, particularly favoring the reducing end, and can degrade dorsal branches with many substitutions because they have low substrate specificity. On the other hand, GH11 enzymes show a preference for internal xylan bonds and unsubstituted xylan chains, acting primarily on the xylose unit in the center of the oligosaccharide chain, hydrolyzing only xylan.

## **3. Biorefinery Concept Applied to Xylooligosaccharides Production**

XOs are a high-value material, and their production from agricultural by-products is a biorefinery concept, as it aims to maximize the utility and value of biomass by its conversion into various products [9]. It is a promising strategy in the production of high-value-added compounds from the hemicellulosic fraction of biomass, promoting the sustainability of the biorefinery model in terms of circular bioeconomy. Integrated lignocellulosic biomass biorefineries can develop bioprocesses to generate bioproducts such as biofuels, bioenergy, enzymes, and chemicals of commercial interest. Cellulose, hemicellulose, and lignin, which are the main components of lignocellulosic biomass, can be used as feedstocks for biorefinery products. Together with XOs, it is possible to produce ethanol, butanol, acetic acid, and citric acid.

## **The Market of Xylooligosaccharides Prebiotics**

The global scenario and market potential of prebiotics have shown that the oligosaccharide market is increasing promptly, reaching a market size of USD 3.3 billion in 2016 and an expectancy of growth by 8.7% (compound annual growth rate—CAGR) by 2026, with profits up to USD 9.4 billion, twice the estimated market in 2020 (USD 4.5 billion). The food industry is the first consumer of the prebiotic market due to the addition in formulation, representing 82% of the market. According to the lower requirement to achieve the prebiotic effect, XOs (dose given of 1.4–2.8 g/day) are considered the most competitively priced prebiotic, with a final cost of less than USD 0.1 per dose. The XOs market has increased in the last two decades, since it seems more efficient in boosting and improving the gut microbiota balance and reducing proinflammatory responses than fructooligosaccharides in diet supplementations, potential features for immunity enhancement and cardiovascular and inflammatory diseases. According to the Market Study Reports, the use of XOs is not exclusively in the food industry; nowadays, XOs can also be applied as an immune-stimulating agent in medicine and as an antioxidant agent, nutraceutical, and cosmetic in the pharmaceutical industry. The market price for XOs prebiotics can vary from USD 25 to 50/kg according to their purity, and the minimum selling price is USD 3430–7500, 4030–8970, and 4840–10,640 per metric ton for 80, 90, and 95% purity.

## **Challenges of Xylooligosaccharides for the Food Industry**

1. There are several crucial challenges for the successful integration of XOs as a functional food supplement in the food industry. These challenges are currently being addressed to

enable the incorporation of XOs into innovative and healthier food products. Some of the common challenges are as follows:

2. Consolidation of scientific studies: While XOs show potential health benefits as prebiotics, further research is needed to establish the specific mechanisms of action, optimal dosage, and potential interactions with other food components. It is essential to have a solid scientific foundation regarding the effects of XOs as prebiotics.
3. Production costs and availability: The cost and availability of XOs can pose a significant challenge for the food industry. The production of XOs involves enzymatic processes and may require specific raw materials, such as lignocellulosic biomass. Developing cost-effective and sustainable production methods for XOs is necessary to make them economically viable for widespread use in the food industry.
4. Organoleptic characteristics and stability: XOs, like other prebiotic ingredients, may have unique flavor and texture characteristics that could impact the sensory profile of food products. Ensuring that XOs do not negatively affect the taste, texture, or overall quality of food products is crucial for consumer acceptance. Moreover, XOs can undergo changes during processing and storage, which may affect stability and shelf life.
5. Quality standards: The food industry must comply with regulatory standards and requirements related to the use of XOs as a food ingredient. Regulatory bodies may have specific guidelines and limits on the concentration and labeling of XOs in food products. Adhering to these standards is necessary to ensure the safe and appropriate use of XOs in food applications.
6. Consumer acceptance: XOs may be relatively new to the market, and consumers may have limited knowledge about their benefits and potential uses. Educating consumers and raising awareness about XOs as a functional food ingredient is crucial for promoting acceptance and adoption. Creating the right conditions based on solid scientific evidence to support the benefits of XOs is important for building consumer confidence, obtaining regulatory approval, and facilitating the seamless integration of XOs into the food market.

## Conclusion

Xylooligosaccharides are classified as emerging prebiotics and they have a plethora of applications, and are termed as novel prebiotics. The unique biological properties of XOS justify the development of new functional foods combining probiotics with prebiotics. The introduction of such functional foods has the potential to ameliorate the quality of life ridden with obesity, hypersensitivity and gastrointestinal disorders, diabetes and cancer. Further research efforts are called for reducing 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 for developing XOS by selecting better substrates, xylanases, LCM pre-treatments and xylan treatments for improving the yield and quality. According to the available literature, the pre-treatment of LCMs with alkali is likely to be the most efficient and safest, and corncobs are the most suitable substrates for XOS production, due to high xylan content and availability.

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