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Review



Unveiling the spectrum of xylooligosaccharides from lignocellulosic biomasses: Production, functional properties, food applications, and market insights

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ABSTRACT

Lignocellulosic agro-industrial residues are a valuable source for oligosaccharide production, supporting the biorefinery concept and advancing innovative production strategies. This review focuses on xylooligosaccharides (XOS) production from various lignocellulosic residues, focusing on xylan as a primary feedstock. It discusses conventional methods (acid hydrolysis, autohydrolysis, enzymatic hydrolysis) and novel approaches (submerged fermentation, solid-state fermentation, deep eutectic solvents), evaluating their benefits, challenges, and optimization potential. The prebiotic activity of XOS, as well as its physicochemical, technological, and sensory properties, underscores its suitability for food applications. The review also explores regulatory frameworks, especially in Brazil, and market insights that influence the commercial value and growth of XOS. Despite growing interest, no standardized production process exists. Moreover, process conditions and feedstock selection remain critical for optimizing yields. Further research is essential to developing more efficient, standardized, and sustainable methods, alongside in vivo studies to ensure regulatory compliance, unlocking the full potential of XOS in the food industry and other sectors.

1. Introduction

Lignocellulosic biomass from agro-industrial waste is the most available renewable resource in the world and an important substrate source for oligosaccharides production, such as xylooligosaccharides (XOS), arabinooligosaccharides (AOS), mannooligosaccharides (MOS), and cellooligosaccharides (COS) (Ávila & Goldbeck, 2022; Ana Flávia Azevedo Carvalho, Neto, da Silva, & Pastore, 2013; Chapla, Pandit, & Shah, 2012; Vikash Kumar & Satyanarayana, 2015). Approximately 1.3 billion tons of lignocellulosic biomass are produced annually worldwide, of which only 3 % is used to generate bioproducts (Pinales-Márquez et al., 2021). Using lignocellulosic agro-industrial waste for oligosaccharide production is economically advantageous due to the cost-effectiveness and widespread availability of these materials, thereby facilitating more comprehensive and organized management of the millions of tons/year of this agricultural waste generated globally (Pinales-Márquez et al., 2021; Poletto et al., 2020). However, some characteristics must be considered when choosing the type of raw material and the end product of interest as these materials vary in composition (Batidzirai et al., 2016).

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Xylooligosaccharides (XOS) are oligomers of xylose traditionally produced using diverse physicochemical and enzymatic processes, either combined or isolated (Samanta et al., 2015), but also adopting unconventional methods, such as direct fermentation (Amorim et al., 2018; Amorim, Silvério, & Rodrigues, 2019), solid-state fermentation (S. S. Reddy & Krishnan, 2016b), and deep eutectic solvents (Isci, Thieme, Lamp, Zverlov, & Kaltschmitt, 2021). Conventional physicochemical methods have limitations, including requirements for elevated temperatures and pressures, leading to the formation of toxic compounds (e.g. hydroxymethylfurfural (HMF), and furfural); thus, enzymatic methodologies have emerged as highly promising alternatives (Akpinar, Ak, Kavas, Bakir, & Yilmaz, 2007; Santibáñez et al., 2021). Implementing controlled process conditions of both physicochemical and enzymatic methods can enhance XOS production efficiency, reduce the generation of undesired byproducts and reduce the production costs (Forsan, Paz Cedeño, Masarin, & Brienzo, 2021). However, it is crucial to acknowledge that XOS production still encounters significant challenges concerning cost and complexity. Despite the high market value of these compounds, identifying optimal production conditions, ensuring an economically viable product with a high yield and suitable functionality remains a substantial challenge (Guido, Silveira, & Kalil, 2019; Poletto et al., 2020).

Due to their promising technological and functional characteristics, XOS are widely used across various industries, including pharmaceuticals (Palaniappan, Antony, & Emmambux, 2021), animal feed (P. K. Gupta, Agarwal, & Hegde, 2012), agriculture, food and cosmetics (Palaniappan et al., 2021). The multifaceted impact of XOS on human health places them as a potential agent against diverse gastrointestinal disorders, insulin resistance, and even cancer, thereby prompting exploration within the pharmaceutical industry for production and commercialization (Martins, Sganzerla, Forster-Carneiro, & Goldbeck, 2023). Their primary beneficial effect includes promoting the balance of the intestinal microbiota. Furthermore, other benefits include preventing the development of diabetes (J. Yang et al., 2015), neurotoxicity, and colon cancer (Ayyappan A. Aachary, Gobinath, Srinivasan, & Prapulla, 2015); enhancing calcium bioavailability by improving absorption; exhibiting antioxidant properties (T. Zhou, Xue, Ren, & Dong, 2018); stimulating the immune response (Zhenping et al., 2013); demonstrating anti-inflammatory and antimicrobial actions (Hansen et al., 2013).

Considered a food ingredient, XOS boasts favorable physicochemical, technological, and sensory attributes for applications across diverse food matrices. Some examples of XOS applications can be found in meat products, baked goods, dairy products, and vegetable protein, as well as acting as sweeteners, emulsifiers, stabilizers, and fat substitutes (Amorim, Silvério, Prather, & Rodrigues, 2019; Palaniappan et al., 2021).

In this context, the present review article provides an extensive overview and critical analysis of different agro-industrial residues as a source of hemicellulose for XOS, AOS, MOS, and COS production, focusing on XOS; elucidating acid and enzymatic hydrolysis as primary production methodologies via optimized treatments, as well as novel unconventional approaches (direct fermentation, solid state, and deep eutectic solvents). Furthermore, the commercial value of XOS will be addressed from different angles, namely by the market and regulatory aspects, particularly focusing on Brazil, offering critical information for both researchers and industry professionals; prebiotic and health-beneficial biological properties; and general commercial applications, highlighting the interesting physicochemical, technological, and sensory attributes of XOS for the food industry, given its growing prominence.

2. Oligosaccharides production from lignocellulosic biomass

Lignocellulosic biomass is the world's most abundant natural and renewable raw material source (Anwar, Gulfraz, & Irshad, 2014). It includes agricultural, forestry, agro-industrial waste and byproducts, and energy crops. Furthermore, activities associated with the plant agribusiness sector are one of the main generators (Araújo, Machado, & Vilarinho, 2019). Most of these materials are disposed of in the environment, often being burned and becoming an environmental problem as there are still no efficient disposal management practices applicable globally (Anwar et al., 2014; Tripathi, Hills, Singh, & Atkinson, 2019). There is a need to explore the potential of these agricultural wastes to obtain bioproducts to add value to the production chain. In this context, there is a vast body of research on producing oligosaccharides from biomass, such as XOS, AOS, MOS and COS.

2.1. Overview of lignocellulosic biomass composition

Biomass typically consists of cellulose, hemicellulose, and lignin, as well as ash, proteins, extractives, phenolic compounds, fats, and fatty acids (Florbela Carvalheiro, Duarte, & Gírio, 2008; Daniel Obed Otieno & Ahring, 2012a). The relative proportions of these constituents vary depending on the biomass type, harvest time, and vegetative stage. Cellulose generally makes up 30–50 %, while hemicellulose and lignin account for 15–35 % and 10–30 %, respectively (Corim Marim & Gabardo, 2021). This variation in structural polysaccharide composition presents a major challenge for efficient biomass conversion and oligosaccharide production (Pinales-Márquez et al., 2021). Table 1 presents the cellulose, hemicellulose, and lignin content of various lignocellulosic materials for oligosaccharide production. Corn cobs can be mentioned due to their high hemicellulose content (38.8 %) (Boonchuay et al., 2018), making them a key raw material for commercial XOS production, used by companies such as Prenexus Health (USA) and Shangdong Longlive Biotechnology (China) (FDA, 2013). Sugarcane bagasse, also rich in hemicellulose (Table 1), is similarly used in XOS production and has GRAS (Generally Recognized as Safe) certification for use in food products.

Other lignocellulosic materials with high hemicellulose content (Table 1), such as pineapple peel (31.8 %) (Banerjee, Patti, Ranganathan, & Arora, 2019), almond shells (32.5 %) (Nabarlatz, Torras, Garcia-Valls, & Montané, 2007), coconut husks (26 %) (Jnawali, Kumar, Tanwar, Hirdyani, & Gupta, 2018), barley hulls (26.8 %) (Parajó, Garrote, Cruz, & Dominguez, 2004), coffee husks (24.6 %) (Ávila, Martins, & Goldbeck, 2021), bamboo (26.6 %) (Ogaki et al., 2009), malt bagasse (29.6 %) (Carvalheiro, Esteves, Parajó, Pereira, & Gírio, 2004), and wheat straw (28.0 %) (Huang et al., 2017), have also attracted interest for oligosaccharide production. Tropical fruit waste, including banana peel (38.4 %) (B. S. Pereira, de Freitas, Vieira, & Brienzo, 2022), guava bagasse (28.3 %) (B. S. Pereira et al., 2022), and orange bagasse (31.0 %) (B. S. Pereira et al., 2022), is another polysaccharide-rich resource for XOS production. Additionally, lower-hemicellulose materials, such as cotton seed (16.1 %) (Teixeira & Borges, 2005), peach palm-peel (20.0 %) (Francielli Vieira et al., 2021), and restaurant food waste (20.6 %) (B. S. Pereira et al., 2022), have been increasingly studied.

The chemical composition of these residues, particularly their hemicellulose polysaccharides (xylan, arabinan, and mannan), determines the type of oligosaccharide that can be produced. Xylan, the main hemicellulose component, consists of a linear chain of xylose units linked by β-(1,4) bonds, with branched side chains of arabinose, galactose, acetyl groups, and glucuronic acids (F. Peng, Peng, Xu, & Sun, 2012; R. Zhang, Lu, Sun, Wang, & Zhang, 2011). Hardwood, with up to 30 % xylan, is highly resistant to hydrolysis due to its dense structure, making it less suitable for XOS production (Rajagopalan, Shanmugavelu, & Yang, 2017). Typically, xylans and arabinans are more abundant than galactans and mannans (Daniel O. Otieno & Ahring, 2012b).

2.2. Oligosaccharide types and production

Table 2 shows the xylan, arabinan, and mannan content as a percentage (dry mass) of some of the lignocellulosic biomasses used to produce oligosaccharides, such as XOS, AOS, MOS and COS. As can be

Table 1
Chemical composition of several lignocellulosic biomasses from agricultural wastes used for oligosaccharides production.

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Agave bagasse	20.8	12.2	17.3	(Pino et al., 2021)
Almond shell	27.0	30.0	36.0	(Álvarez et al., 2019)
Bamboo	40.4	21.6	25.9	(Xiao et al., 2013)
Banana peel	20.7	17.3	31.6	(B. S. Pereira et al., 2022)
Banana pseudo-stem	60.8	19.6	17.2	(B. S. Pereira et al., 2022)
Barley hull	21.4	26.8	19.2	(Parajó et al., 2004)
Brewer's spent grain	21.9	20.6	21.7	(F. Carvalheiro et al., 2004)
Coconut husk	34.1	26.0	32.6	(Jnawali et al., 2018)
Coffee husk	27.7	24.6	35.5	(Ávila et al., 2021)
Corn straw	40.0	25.0	17.0	(Saha, 2003; Santibáñez et al., 2021)
Corncob	35.0	34.8	14.0	(R. Yang, Xu, Wang, & Yang, 2005)
Cotton seed	18,0	16.1	–	(Teixeira & Borges, 2005)
Cotton stalks	35.6	21.4	27.8	(Akpinar et al., 2007)
Hazelnut shell	30.0	23.0	38.0	(Álvarez et al., 2019)
Malt extract	17.9	21.8	19.9	(da Silva Menezes, Rossi, & Ayub, 2017.)
Mongo seed shell	45.6	18.3	29.4	(Monteiro et al., 2021)
Nutshell	37.0	22.0	36.0	(Álvarez et al., 2019)
Orange bagasse	28.9	31.7	9.52	(B. S. Pereira et al., 2022)
Peanut shell	20.9	19.3	42.7	(Rico, Gullón, Alonso, Parajó, & Yáñez, 2018)
Peach palm-peel	36.0	20.0	20.0	(Francielli Vieira et al., 2021)
Peach palm-inner	34.2	19.0	23.0	(Francielli Vieira et al., 2021)
Pigeon pea	42.7	18.3	2.31	(Samanta et al., 2013)
Pine nutshell	31.0	25.0	38.0	(Álvarez et al., 2019)
Pineapple peel	20.9	31.8	10.4	(Banerjee et al., 2019)
Rice husk	37.4	11.2	25.5	(da Silva Menezes et al., 2017.)
Soybean husk	38.3	19.5	6.5	(da Silva Menezes et al., 2017)
Soybean fiber	4.3	22.2	4.3	(Pereira et al., 2018)
Sugarcane bagasse	40.0–43.0	25.0–28.0	23.0–28.0	(Poletto et al., 2020)
Sugarcane straw	39.4	29.8	21.8	(Ávila et al., 2021)
Sunflower stem	42.3	–	26.5	(Akpinar et al., 2009)
Tobacco stalk	40.4	–	27	(Akpinar et al., 2009)
Wheat straw	36.6	22.7	22.9	(Huang et al., 2017)

- No data found.

seen in Table 2, concerning the xylan content, some unexplored raw materials warrant more attention for XOS production, such as cottonseed, which has almost twice as much xylan (64.7 %) (H.-J. Sun, Yoshida, Park, & Kusakabe, 2002) as corn cob (31.9 %), the most common raw material used for this purpose (Ayyappan Appukuttan Aachary & Prapulla, 2009).

On the other hand, higher levels of arabinan, a polysaccharide consisting mainly of arabinose (Smestad Paulsen & Barsett, 2005), are desired for arabinoxylooligosaccharides (AXOS) production. One of the main constituents of the structure of cereals is arabinoxylan, made up of xylose residues, some of which are replaced by arabinose residues (Andersson & Åman, 2000). It can be seen that malt bagasse is a very attractive raw material and is already indicated for producing AXOS (Amorim, Silvério, & Rodrigues, 2019), with arabinan contents of 5.1 and 9.0 % (Table 2), corresponding to malt bagasse obtained from different batches or processing conditions. The value of 5.1 % represents arabinan content in malt bagasse derived from conventional brewing processes, while 9.0 % is associated with malt bagasse from a modified or optimized brewing method aimed at enhancing polysaccharide retention. The variation between these two values can be attributed to factors such as the raw material's source, brewing process parameters (e.g., temperature, pH, and enzymatic activity during mashing), and storage conditions. These parameters influence the retention and extraction of arabinoxylan and their associated arabinan fractions in the malt bagasse. In addition, higher arabinan levels, as observed in the 9.0 % sample, suggest greater potential for producing arabinoxylooligosaccharides (AXOS), given that arabinan contributes arabinose residues essential for the AXOS structure. This highlights the importance of optimizing malt bagasse processing to enhance its suitability for AXOS production.

Mannans are polysaccharides found in plants consisting of mannose units joined by β-(1,4) glycosidic bonds (Soni & Kango, 2013) and can be classified as α- and β-MOS (Ayimbila & Keawsompong, 2022). Partial hydrolysis of mannan by enzymatic or chemical methods produces mannooligosaccharides (MOS), short-chain carbohydrates formed with 2 to 10 mannose residues (Chiyanzu, Brienzo, García-Aparicio, & Görgens, 2014; Tester & Al-Ghazzewi, 2013). Concerning MOS production, plant gums are the main materials used to produce these oligomers, however, the extraction processes are expensive (Srivastava, Panwar, Prashanth, & Kapoor, 2017; Yamabhai, Sak-Ubol, Srila, & Haltrich, 2016). Some lignocellulosic residues have a high mannan content and are low-cost alternatives for β-MOS production, such as palm kernel cake with 43.2 % mannan (Y. X. Li, Yi, Liu, Yan, & Jiang, 2018) (Table 2). On the other hand, α-MOS can be obtained by cleaving α-(1, 6) bonds in the yeast cell wall (Orlean, 2012). Coffee grounds are waste that not only have a high mannan composition but also a high galactan composition, known as a waste rich in galactomannans (40–50 % mannan and 30–35 % galactan) (Getachew & Chun, 2017). They release non-digestible MOS after hydrolysis and are widely exploited as prebiotics (Nguyen, Cho, Lee, & Bae, 2019). Table 2 shows a high content of mannan (34.3 %) and galactomannan (49.9 %) in the study by Gu et al. (Gu et al., 2020), confirming this information. Copra flour, a coconut kernel residue, also has high galactomannan content and is a suitable source of MOS, as observed in the study by Ghosh et al. (Ghosh, Verma, Tingirikari, Shukla, & Goyal, 2015), with around 74 % galactomannan (Table 2).

Other oligosaccharides obtained from biomass are attracting interest in the food industry due to their prebiotic potential, such as COS (Ávila & Goldbeck, 2022). These non-digestible short-chain oligosaccharides are defined as linear oligomers with 2 to 6 glucose units joined by a β-(1,4) glycosidic bond (Otsuka et al., 2004), water-soluble and capable of promoting the growth of probiotic bacteria (Karnaouri, Matsakas, Krikigianni, Rova, & Christakopoulos, 2019). Some examples of lignocellulosic biomass already used for COS production include sugarcane straw (Ávila, de Mélo, & Goldbeck, 2023; Barbosa et al., 2020), coffee husks (Ávila et al., 2023), and wood waste (Karnaouri et al., 2019).

Table 2
Composition of the xylan, arabinan, and manan of different lignocellulosic residues used for oligosaccharide production.

Biomass	Xylan (%)	Glucan (%)	Arabinan (%)	Mannan (%)	Galactan (%)	Galactomannan (%)	Ref.
Banana pseudo-stem	14.0	37.6	5.8	–	–	–	(Freitas, Terrone, Carmona, & Brienzo, 2020)
Barley hull	26.8	–	5.7	–	–	–	(Parajó et al., 2004)
Brewer's spent grain	10.3	–	5.1	–	–	–	(Amorim, Silvério, & Rodrigues, 2019)
Chestnut shell	10.4	20.6	2.9	–	–	–	(Gullón et al., 2018)
Coffee husk	20.3	–	–	–	–	–	(Ávila et al., 2021)
Copra meal	–	–	–	–	–	74.0	(Ghosh et al., 2015)
Corncob	31.3	34.4	3.0	–	–	–	(Parajó et al., 2004)
Cotton seed	64.7	–	–	–	–	–	(H.-J. Sun et al., 2002)
Cotton stem	19.8	–	–	–	–	–	(Akpinar et al., 2009)
Corn straw	21.5	–	–	–	–	–	(Santibáñez et al., 2021) (Saha, 2003)
Palm kernel cake	–	–	–	43.2	–	–	(Y. X. Li et al., 2018)
Spent coffee ground	–	9.4	2.3	34.3	15.6	49.9	(Gu et al., 2020)
Soybean straw	11.4	–	1.0	1.7	1.8	–	(Wan, Zhou, & Li, 2011)
Soybean fiber	15.1	–	7.0	–	–	–	(G. F. Pereira et al., 2018)
Sunflower stem	19.1	–	–	–	–	–	(Akpinar et al., 2009)
Sugarcane bagasse	21.9	–	1.9	0.8	0.5	–	(de Figueiredo, Carvalho, Brienzo, Campioni, & de Oliva-Neto, 2017)
Sugarcane straw	22.6	–	5.8	–	–	–	(Ávila et al., 2021)
Wheat straw	25.7	41.1	2.2	–	–	–	(J. Xu et al., 2019)

- No data found.

2.3. Challenges and opportunities in oligosaccharide production

The selection of suitable lignocellulosic biomass is critical for the successful production of oligosaccharides, such as xylo-oligosaccharides (XOS) and arabinoxylo-oligosaccharides (AXOS), as different biomass types present different challenges and advantages. Variations in cellulose, hemicellulose, and lignin content directly affect extraction and conversion efficiency, where high lignin levels often require more

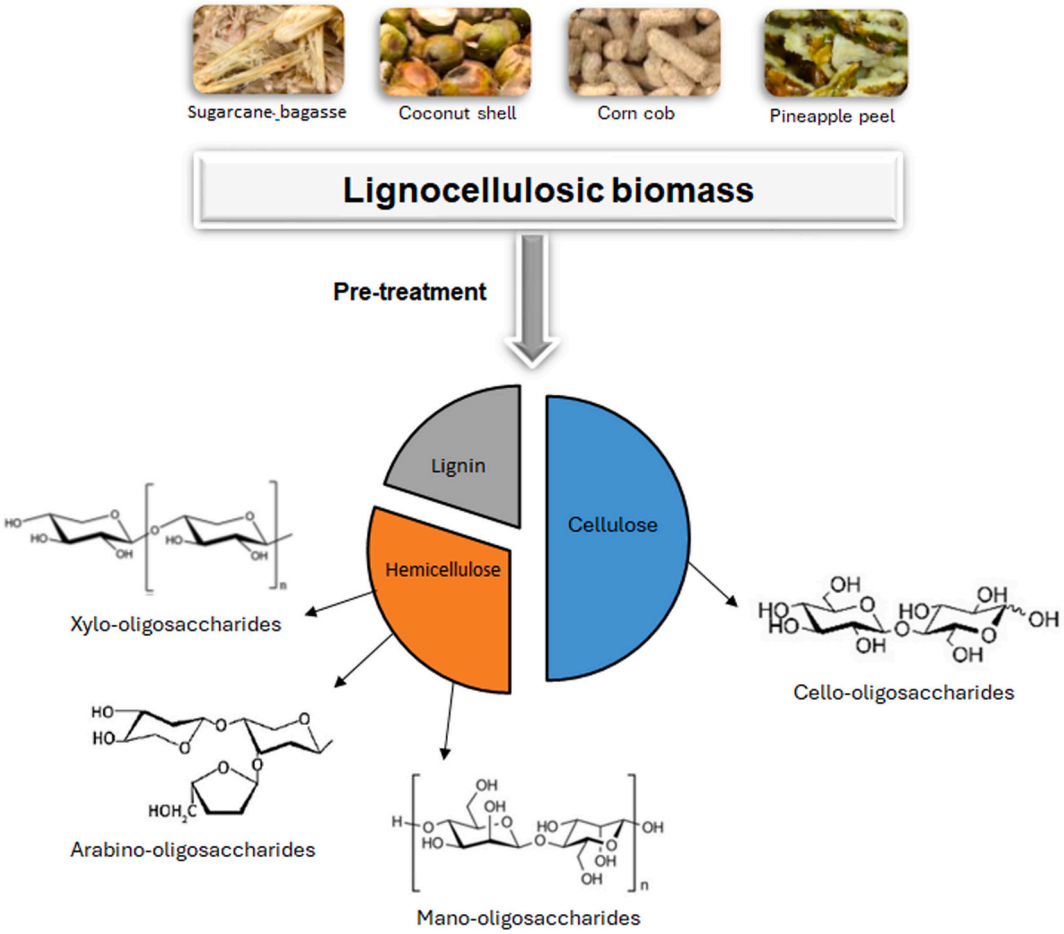


Fig. 1. Oligosaccharides from lignocellulosic biomass.

intensive and costly pretreatments (Mussatto & Teixeira, 2010). Additionally, regional and seasonal availability, as well as acquisition and transportation costs, may impact economic feasibility (Valipour et al., 2024). Another challenge is the formation of inhibitory by-products, such as furfural and acetic acid, during chemical or thermal pretreatment, which can hinder subsequent enzymatic hydrolysis (Florbela Carvalheiro et al., 2008). Conversely, agro-industrial residues, including sugarcane bagasse, cereal husks, and fruit peel, offer significant opportunities for value addition and circular economy practices (Ravindran & Jaiswal, 2016). Innovative technologies, such as green pretreatments, can enhance oligosaccharide recovery even from recalcitrant biomass, while bioactive compound-rich residues, such as maize and wheat, provide the potential for high-value functional products with prebiotic properties (Amorim, Silvério, Prather, & Rodrigues, 2019). The global diversity of lignocellulosic resources enables biomass selection suited to local conditions and production targets, underscoring the need for research integrating biomass characteristics with sustainable processing technologies.

3. Xylooligosaccharides (XOS)

XOS are oligomers of xylose units (2−10) linked by β-(1,4) glycosidic bonds (Fig. 1). They are indigestible by humans and act as prebiotics, selectively promoting beneficial intestinal microbiota and offering various health benefits (Finegold et al., 2014; Palaniappan et al., 2021). The degree of polymerization (DP) of XOS ranges from 2 to 10 and includes xylobiose (DP2), xylotriose (DP3), xylotetraose (DP4), xylopentose (DP5), xylhexose (DP6), and higher oligomers (Ana Flávia Azevedo Carvalho et al., 2013; Samanta et al., 2015). The DP is mainly influenced by the production method. Chemical hydrolysis and autohydrolysis are aggressive and non-selective processes that cleave larger hemicellulose structures, yielding XOS with higher DP (DP6–DP10). In contrast, enzymatic methods are highly specific, and their activity is hindered by side chains and branching in xylan, resulting in lower DP XOS (DP2–DP5).

Thus, XOS with higher DP (DP6–DP10) are commonly produced by chemical hydrolysis and autohydrolysis (Kaprelyants, Zhurlova, Shpyrko, & Pozhitkova, 2017), whereas enzymatic processes primarily yield lower DP XOS (DP2–DP5) (A. F. Carvalho et al., 2015; Kaprelyants et al., 2017). Additionally, variations in XOS structure, DP, and substitution patterns depend on the biomass source and production process (Amorim, Silvério, Prather, & Rodrigues, 2019; Samanta et al., 2015).

XOS are naturally found in limited quantities in vegetables, fruit, milk, bamboo shoots, and honey but are economically unviable for large-scale production and purification (Vázquez, Alonso, Domínguez, & Parajó, 2000). Agro-industrial residues rich in xylan are ideal substrates for industrial-scale XOS production, adopting chemical, physical, or enzymatic methods, either individually or in combination (Kaprelyants et al., 2017; Palaniappan et al., 2021).

Hemicellulose conversion into XOS depends on the xylan source. For instance, xylan chains may have side groups such as α-D-glucopyranosyl acid (uronic acids) or its 4-*O*-methyl derivative, acetyl groups, or arabinofuranosyl residues, leading to branched XOS with diverse biological properties (Ayyappan Appukuttan Aachary & Prapulla, 2011; Mano

Table 3
XOS production from several lignocellulosic biomass by chemical (acid and alkaline hydrolysis) and autohydrolysis ways.

Treatment	Biomass type	Process conditions	Enzymatic hydrolysis	XOS yield (mg/g biomass)	References
Acid	Sugarcane bagasse	Sulfuric acid diluted: 100 °C/ 30 min	α-L-arabinofuranosidase and endoxylanase	115.0	(Ávila et al., 2020)
		Gluconic acid 5 %: 150 °C/60 min	not applied	140.9	(X. Zhou et al., 2019)
		Sulfuric acid 0.5 %: 190 °C/5 min and steam explosion	not applied	82.5	(Ana Flavia Azevedo Carvalho et al., 2018)
		Sulfuric acid diluted 2 %: 79.55 °C/35 min	not applied	25.8**	(Forsan et al., 2021)
	Sugarcane leaves	Acetic acid diluted 2 %: 180 °C/35 min	not applied	6.2**	(Forsan et al., 2024)
		Sulfuric acid diluted 2 %: 79.55 °C/35 min	not applied	19.9**	(Forsan et al., 2024)
	Beech wood	Sulfuric acid 0.1 M: 90 °C/2 h	not applied	54***	(Beckendorff et al., 2023)
	Palm leaf bagasse	Diluted nitric acid 0.1 %: 60 °C/12 h	Commercial cellulase	174.7	(Mazlan, Samad, Wan Yussof, Saufi, & Jahim, 2019)
	Corn cobs	Gluconic acid:154 °C/47 min	Cellulases	180.0	(Han et al., 2020)
		Acetic acid:150 °C/30 min	not applied	139.8	(H. Zhang et al., 2017)
Alkaline	Sugarcane bagasse	KOH 5 %: 121 °C/30 min	Endoxylanase	32.0	(de Figueiredo et al., 2017)
	Pineapple peel	NaOH 15 %: 121 °C/ 1.5 h	Endoxylanase (Megazyme)	82.0	(Banerjee et al., 2019)
	Coconut shell	NaOH 20 % and steam explosion/60 min	Crude enzyme extract	63.0	(Ayyappan Appukuttan Aachary & Prapulla, 2009)
	Corn cobs	NaOH 2 % at room temperature overnight	Endoxylanase from *A. oryzae* MTCC 5154	115.0	(Boonchuay et al., 2018)
Auto hydrolysis	Sugarcane bagasse	162 °C/75 min	not applied	53.3	(Dias et al., 2023)
		200 °C/10 min	Cellulase	52.9*	(Zhang et al., 2018)
	Banana peel	160°/15 min	not applied	32.3***	(B. S. Pereira et al., 2023)
	Guava bagasse	172,4 °C/35 min	not applied	8.2***	(B. S. Pereira et al., 2023)
	Orange bagasse	130 °C/35 min	not applied	66.1***	(B. S. Pereira et al., 2023)
	Sugarcane straw	177 °C/65 min	not applied	96.0	(Dias et al., 2023)
	Almond shell	200 °C/15 min	Endoxylanase from *T. lanuginosus*	110.0	(Singh, Nadar, Muir, & Arora, 2019)
	Peanut shells	210 °C	Not applied	3.0	(Rico et al., 2018)
	Hazelnut shells	190 °C	not applied	115.9	(Surek & Buyukkileci, 2017)
	Chestnut shell	180 °C	not applied	57.0	(Gullón et al., 2018)
	Corn cobs	Auto hydrolysis with dilute acid: 135 °C/30 min	Xylanase from *P. corylophilum*	235.5	(R. Yang et al., 2005)

*Yield expressed in mg of XOS/g of Xylan; **yield expressed in g/L and ***yield expressed in % conversion of xylan into XOS.

et al., 2018). These substitutions influence fermentation by Bifidobacterium spp. and Lactobacillus spp., with linear or branched arabinose XOS (AXOS) fermenting faster than XOS with acetyl or methylglucuronic acid groups (Falck et al., 2013; Kabel, Kortenoeven, Schols, & Voragen, 2002).

3.1. Processes for producing XOS from lignocellulosic biomass

Xylo-oligosaccharides (XOS) can be produced using various methods, in which two-stage production combining chemical, physical, and enzymatic processes (Tables 3 and 4) is among the most common strategies (Santibáñez et al., 2021). Hydrothermal and acid treatments, including autohydrolysis and dilute acid hydrolysis, effectively hydrolyze hemicellulose into oligomers and monosaccharides but require significant optimization to limit monosaccharide formation and sugar degradation.

Large-scale XOS production faces challenges such as high enzyme costs, the need for efficient purification to remove byproducts, and the formation of inhibitory compounds such as furfural, HMF, and phenolic derivatives, which can reduce yield or demand additional processing steps (Sonkar, Gade, Mudliar, & Bhatt, 2023). Emerging approaches (Fig. 2), including direct and solid-state fermentation and the use of deep eutectic solvents, show promise in overcoming these limitations and enhancing process efficiency (Santibáñez et al., 2021).

Table 4
XOS production via combination of pre-treatments and enzymatic hydrolysis of lignocellulosic biomass.

Biomass type	Conditions	XOS yield	References
Sugarcane bagasse	Ionic liquid and dilute sulfuric acid pre-treatment and commercial Endo-1,4-xylanase and α-L-arabinofuranosidase	115.0 mg/g xylan	(Ávila et al., 2020)
	Pre-treatment with aqueous ammonia; Xylanase from *Bacillus subtilis* KCX006 and arabinofuranosidase	139.7 mg/g biomass	(Shyam Sunder Reddy & Krishnan, 2016a)
	Enzyme cocktail containing xylanases and accessory enzymes to complete the hydrolysis of xylan	19.6 g/L	(Bueno, de Freitas, & Brienzo, 2023)
Bean culm	Endo-β-1,4-xylanase: 50 °C/ 2 h	105 mg/g	(I. J. Kim et al., 2023)
Cottonseed hulls	Endo-β-1,4-xylanase: 50 °C/2 h	45 mg/g	(Dukare et al., 2024)
Pineapple bagasse	Recombinant xylanase: 50 °C, pH 5.2, 180 min, 300 rpm	2.70 mg/mL	(Chaudhary et al., 2023)
Pineapple stem	Recombinant xylanase: 50 °C, pH 5.2, 180 min, 300 rpm	2.37 mg/mL	(Chaudhary et al., 2023)
Rice husks	Xylanase from recombinant *Aspergillus brazilianus* BLf	37.25 mg/g biomass	(da Silva Menezes et al., 2018)
	Xylanase from recombinant *Aspergillus nidulans* XynC A773	75.92 mg/g biomass	(da Silva Menezes et al., 2018)
Soybean fiber	Endo-β-1,4-xylanase / 50 °C, pH 6.0, 9 h	17.35**	(Kaur et al., 2024)
	Xylanase and arabinofuranosidase from *Aspergillus nidulans* A773	288.6 mg/g biomass	(G. F. Pereira et al., 2018)
	Endoxylanase/ α-arabinofuranosidase: 50 °C, pH 4.75, 9 h	180 mg xylobiose/g	(Yegin, 2023)
Wheat straw	Alkaline pre-treatment and *Bacillus halodurans* S7 endoxylanase A mutated at K80R	360.0 mg/g xylan	(Faryar et al., 2015)
	Endo-β-1,4-xylanase: 50 °C, pH 4.8, 150 rpm, 48 h	7.10 mg/mL	(Lymperatou et al., 2023)
Corn cobs	Alkaline pre-treatment and Xylanase from *Aspergillus foetidus* MTCC 4898	673.0	(Chapla et al., 2012)
	Acid and alkaline pre-treatment and Xylanase from *Bacillus aerophilus* KGJ2	114.0 mg/g xylan	(Gowdhaman & Ponnusami, 2015)
	Immobilization of endoxylanase from *Bacillus subtilis*	515 mg/g xylan	(B. Wu et al., 2019)
	Pre-treatment with steam explosion and acid electrolyzed water and recombinant Xylanase from *Paenibacillus barengoltzii*	750 mg/g xylan	(Xueqiang Liu et al., 2018)
	Fusion enzymes: 40 °C, 200 rpm, 72 h	6.91 mg/g biomass	(Shao et al., 2024)

3.1.1. Auto-hydrolysis

XOS can be synthesized through auto-hydrolysis (Table 3), involving xylan deacetylation at elevated temperatures (150–220 °C) and pressures in the presence of water vapor or liquid water, which leads to acetic acid formation and catalyzes xylan hydrolysis. This method preserves XOS structure, increases yield, and reduces polymerization degree, without using corrosive chemicals (Moure, Gullón, Domínguez, & Parajó, 2006). However, it requires high energy input, affecting sustainability depending on the energy source (Daniel Obed Otieno & Ahring, 2012a). Furthermore, the resulting hemicellulose hydrolysate, in addition to oligosaccharides, contains by-products such as monosaccharides, acetic acid, HMF, furfural, formic acid, levulinic acid and phenolic compounds, which reduce the prebiotic properties of XOS (Ayyappan Appukuttan Aachary & Prapulla, 2011). The degree of polymerization of XOS obtained through autohydrolytic and chemical methods is uncertain, often leading to undesirable by-products such as hydroxymethylfurfural (HMF) and furfural. These methods require specialized equipment to achieve high temperatures and pressures, as well as high concentrations of alkalis or acids (Fuso et al., 2022). Compounds, such as HMF and furfural inhibit microbial growth and fermentation (Rasmussen, Sørensen, & Meyer, 2014; L. Zhang, Zhang, Zhang, & Jiang, 2021), while formic and levulinic acids lower the pH and further degrade sugars, affecting enzyme function and microbial growth (K. Kumar, Pathak, & Upadhyayula, 2020; Rasmussen et al., 2014). Phenolic compounds damage cell membranes and enzymes (Rasmussen et al., 2014), and acetic acid, although less toxic, disrupts fermentation and acidifies the medium (Murlidhar Sonkar, Savata Gade, Bokade, Mudliar, & Bhatt, 2021; Xiao et al., 2013). Therefore, hydrolysate purification using chromatographic or membrane techniques is necessary, increasing production costs (Santibáñez et al., 2021; Vázquez et al., 2000).

Regarding XOS yields (Table 3), some works deserve to be highlighted, such as the study of Dias et al. who researched sugarcane bagasse and straw, reporting XOS yields of 53.3 mg/g (bagasse at 162 °C for 75 min) and 96 mg/g (straw at 177 °C for 65 min), demonstrating that autohydrolysis is a viable alternative for XOS production without additional chemicals (Dias, Neto, Brienzo, de Oliveira, & Masarin, 2023). Similarly, Pereira et al. evaluated various agro-industrial residues subjected to autohydrolysis, obtaining significant yield variations: banana peel (32.3 mg/g at 160 °C for 15 min), guava bagasse (8.2 mg/g at 172.4 °C for 35 min), and orange bagasse (66.1 mg/g at 130 °C for 35 min), highlighting the direct influence of biomass composition on process efficiency and showing,by this way, the potential and drawbacks of this procedure (B. S. Pereira, de Freitas, Masarin, & Brienzo, 2023).

3.1.2. Dilute acid hydrolysis

Acids have been frequently used for XOS production (Table 3) due to their rapid and easy application (Akpinar, Erdogan, & Bostanci, 2009). In dilute acid treatments, hemicellulose is the primary polysaccharide subjected to hydrolysis, resulting in a xylose-rich solution with low lignin content (Geddes et al., 2010). For acid hydrolysis, inorganic or

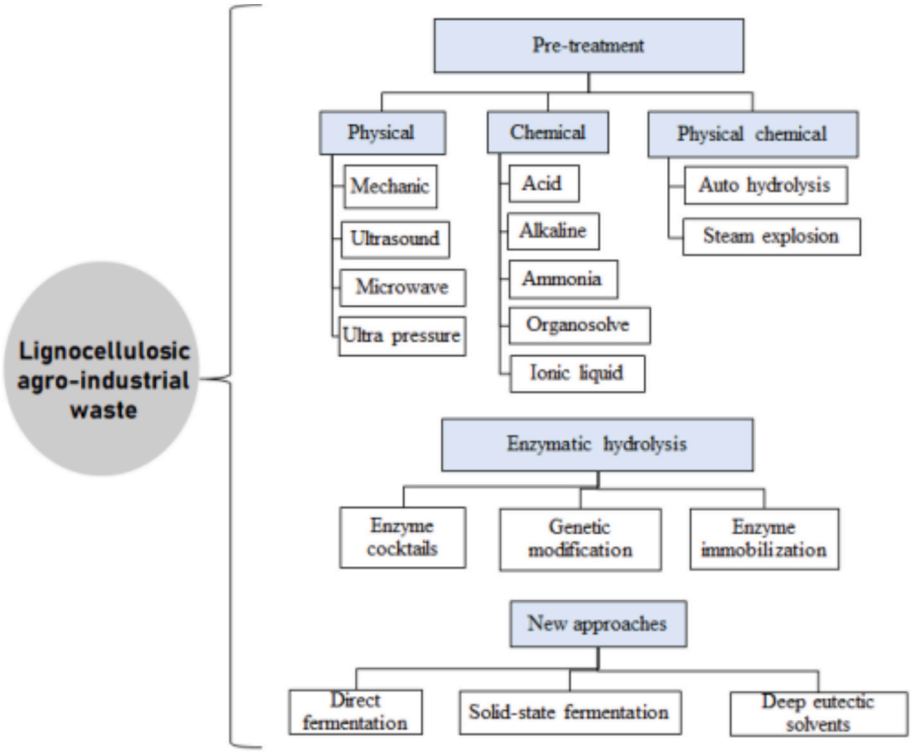


Fig. 2. Strategies for producing XOS from lignocellulosic agroindustrial waste.

organic acids, along with specific temperature and pressure conditions, lead to random cleavage of β-1,4 glycosidic bonds in the xylan chain, releasing not only monomeric xylose but also oligosaccharides of varying degrees of polymerization. However, sugar degradation compounds, such as HMF and furfural can also be produced (de Freitas, Carmona, & Brienzo, 2019). In addition to acid hydrolysis, other chemical pretreatment methods can impact XOS integrity. Harsh conditions may cause partial hydrolysis or degradation, altering molecular weight distribution and potentially forming monosaccharides or by-products. These changes can affect XOS functionality, including fermentability and prebiotic activity. Thus, optimizing pretreatment conditions is crucial to maximize yield while preserving structural integrity and prebiotic potential (Gruening de Mattos et al., 2024).

Studies employing more severe acid concentration, reaction time, and temperature conditions are commonly found in the literature. Under such conditions, low yields of oligosaccharides are obtained alongside monosaccharides, indicating that acid treatment is more conducive to xylose than to XOS production (Qing, Li, Kumar, & Wyman, 2013; Samanta et al., 2013). Nevertheless, by optimizing acid concentrations and reaction times, it has been demonstrated that acid treatment can be an efficient pre-treatment method for subsequent enzymatic hydrolysis in the biomass conversion (Corim Marim & Gabardo, 2021; Qing et al., 2013). Forsan et al. demonstrated the effectiveness of controlled conditions, utilizing dilute acids, such as sulfuric and acetic acid in an optimized process with sugarcane bagasse for XOS production (Forsan et al., 2021). This study successfully converted xylan into XOS under milder conditions and identified optimal experimental conditions to minimize xylose and degradation product formation, consequently reducing subsequent product purification steps. In a study conducted by Forsan et al. (Table 3) the best conditions for XOS production involved pre-treatment with 2 % (*w/v*) sulfuric acid at 79.55 °C for 35 min, resulting in a xylan conversion rate of 90.13 %, and an XOS concentration of 25.85 g/L (Forsan et al., 2021).

Table 3 presents a range of acids and their corresponding process conditions utilized in XOS production, including dilute sulfuric and nitric acid. Dilute acids offer advantages over concentrated forms due to their lower concentrations, which reduce reagent wastage and equipment corrosion (Amorim, Silvério, Prather, & Rodrigues, 2019; H. Chen et al., 2017). Table 3 also shows the use of acids, not widely investigated for producing XOS, such as gluconic and acetic acid. Regarding the potential use of gluconic acid in XOS production, a yield of 140.9 mg/g from sugarcane bagasse was obtained (X. Zhou, Zhao, Zhang, & Xu, 2019). Other studies have also shown good yields but with other raw materials, as reported by Han et al., who obtained 180 mg XOS/g from corn cobs (Han, Cao, Zhou, & Xu, 2020); and Dai et al., who yielded 503 mg XOS/g from sorghum straw (L. Dai et al., 2022). In addition to gluconic acid, the use of acetic acid has recently been highlighted for XOS production from sugarcane bagasse (Forsan et al., 2021; X. Zhou et al., 2019), and corn cobs (H. Zhang, Xu, & Yu, 2017), respectively. The great potential of acetic acid for XOS production is due to its moderate hydrolysis, higher oligomer formation rate, and lower formation of degradation products (X. Zhou et al., 2019).

Novel studies have investigated pre-treatment methods that involve more economical and environmentally friendly process conditions, as shown in Table 3. For example, in the study by Wen et al., two pre-treatment stages with acetic acid and sodium chlorite were conducted on poplar hemicellulosic biomass, and an XOS yield of 40 mg/g was obtained, with 400 mg/g of glucose (Zhang et al., 2020a). In parallel, Zhang et al. based their proposal on chemical agents (X. Zhang, Zhang, Lei, Yang and Jiang, 2020a). Under moderate conditions (175 °C for 30 min), they used only seawater to pre-treat sugarcane bagasse and produce XOS, obtaining a maximum yield of 671.2 mg/g of xylan. It should be noted that steam explosion and hydrothermal pre-treatments generally favor the deacetylation of thermolabile acetyl groups attached to the hemicellulose structure and cause the release of acetic acid. As acetic acid is weak compared to inorganic acids, partial hydrolysis of xylan occurs, and the resulting pre-hydrolysates are predominantly XOS. Regarding this same principle, Yao et al. confirmed that the pH of the medium plays a decisive role in breaking the bonds of the lignin carbohydrate complex (Yao et al., 2018).

More recently several others studies have been carried out, e.g., Forsan et al. who investigated XOS production from sugarcane leaves

using acid hydrolysis with diluted acetic acid (2 %) at 180 °C for 35 min and diluted sulfuric acid (2 %) at 79.55 °C for 35 min, obtaining yields of 6.2 mg/g and 19.9 mg/g, respectively (Forsan, Schmatz, Masarin, & Brienzo, 2024). These results indicate that sulfuric acid was more effective for XOS extraction than acetic acid. Whereas, Beckendorff et al. studied acid hydrolysis of beechwood using sulfuric acid (0.1 M) at 90 °C for 2 h, achieving a yield of 54 mg/g (Beckendorff, Lamp, & Kaltschmitt, 2023). This significantly higher value compared to Forsan et al. (2024) may be attributed to differences in lignocellulosic composition or treatment conditions.

When using chemical treatments to produce XOS, the main products of interest are XOS with a lower degree of polymerization. The ratio of polysaccharides to monosaccharides is important in assessing the quality of the hydrolysate, as it is an indicator of the efficiency of the pretreatment process. Operating conditions must, therefore, be controlled to achieve an economically viable process in terms of the yield of the product of interest and lower production of sugar degradation compounds, which act as fermentation inhibitors, reducing the effectiveness of the treatment (Akpinar et al., 2009; Behera, Arora, Nandhagopal, & Kumar, 2014; Chiaramonti et al., 2012).

3.1.3. Alkaline as biomass pretreatment

Alkaline pretreatments are typically carried out using sodium hydroxide, sodium carbonate, calcium hydroxide, and other alkalis, operating over a wide range of conditions, with temperatures ranging from ambient to over 200 °C (J. S. Kim, Lee, & Kim, 2016; Modenbach & Nokes, 2012). An important advantage of using alkaline biomass pretreatments is that specialized equipment is not required, as alkaline reagents generally do not cause corrosion, such as diluted acids. However, in some cases, the cost of reagents and process duration can be limiting factors, particularly when mild temperature and pressure conditions are used (Xu & Sun, 2016). This approach effectively increases the material's surface area by swelling the particles, enhancing carbohydrate accessibility to enzymes and reducing both the degree of polymerization and crystallinity of the cellulosic fraction (Modenbach & Nokes, 2012). Under strong alkaline concentrations, dissolution, terminal group "peeling," alkaline pretreatment dissolve/solubilize polysaccharides such as hemicelluloses. Alkaline hydrolysis involves the saponification of intermolecular ester bonds that cross-link xylan hemicelluloses and other components, increasing the porosity of lignocellulosic biomass as these cross-links break down (J. K. Xu & Sun, 2016).

The primary effect of alkaline pretreatment is biomass delignification, which facilitates subsequent enzymatic hydrolysis of cellulose in second-generation ethanol biorefineries. Although this is the most commonly reported application, alkaline pretreatment can be used to obtain different products from lignocellulosic biomass (Nascimento et al., 2016). The hemicellulose fraction can be extracted under alkaline conditions, where ester bonds between lignin and carbohydrates are broken. Then, the selection of a hemicellulose extraction method depends significantly on the intended use of the recovered hemicellulose (M. Brienzo, Siqueira, & Milagres, 2009; Worku, Bachheti, Bachheti, Milessi and Chandel, 2024). For instance, for ethanol production, complete hydrolysis of hemicellulose into monomeric sugars is preferred, thus treatments involving acids are more appropriate.

Conversely, when aiming to obtain the oligomeric or polymeric forms of hemicellulose components, alkaline extraction techniques, possibly coupled with a refining step (e.g., sodium chlorite, ultrasound, or microwaves), yield superior outcomes (F. Peng et al., 2012; P. Peng & She, 2014).

When alkaline treatment is used, the xylan chains are preserved with a higher molar mass, unlike other treatments, such as acids, which depolymerize a large part of the hemicellulose, resulting in monomers (Sixta & Schild, 2009).

Alkaline methods can correspond to an interesting alternative for xylooligosaccharides (XOS) production, associated to a subsequent xylan hydrolysis. Depending on the process conditions (such as alkali concentration, reaction time, and temperature), hemicellulose can be extracted in soluble macromolecular forms, which can subsequently be enzymatically hydrolyzed in a controlled manner to obtain XOS (Khatudomkiri et al., 2018). As xylan is extracted with lignin in alkaline processes, a subsequent fractionation step is necessary, which may involve precipitation at different pH values and using solvents such as ethanol, or membrane separation processes. For instance, an alkaline pretreatment was evaluated for xylan extraction from sawdust of the Chilean *Nothofagus* species (Lehuedé et al., 2023). The authors tested different NaOH concentrations for sawdust soaking, after which the mixture was autoclaved at 121 °C at different times. The extracted xylans were purified by reducing the pH to 5 and precipitating with ethanol or through ultrafiltration. They were then enzymatically hydrolyzed with xylanases to produce XOS. This study found that using lower alkali concentrations combined with membrane separation resulted in xylan with higher purity, which favors XOS production.

In another study (Shuai Zhao et al., 2021), a mild pretreatment process for sugarcane bagasse using potassium hydroxide, combined with subsequent phosphoric acid pretreatment and enzymatic hydrolysis of xylan with endoxylanases, resulted in 7.15 g of XOS per 100 g of bagasse. The optimized conditions determined by the authors corresponded to 1.9 % KOH at 121 °C and 1 bar for 50 min, followed by 3.9 % H_3PO_4 at 54 °C for 44 min.

3.1.4. Enzymatic hydrolysis

Enzymatic hydrolysis for XOS production (Table 4) can occur in several ways: enzymatic action on lignocellulosic material; a combination of chemical and physical treatments due to the inherent resistance of xylan-lignin structures to xylanolytic enzymes; and the use of immobilized enzymes cXZgOsyz (Amorim et al., 2020; Huang et al., 2017; Jain, Kumar, & Satyanarayana, 2015).

Enzymatic processes for hydrolysis of the xylan chain to produce XOS are advantageous due to the high purity of the product obtained without forming undesirable compounds, in addition to producing XOS with a low DP (A. F. Carvalho et al., 2015; Poletto et al., 2020). Although it allows to achieve competitive yields, the process is slow, and the costs are high (A. F. Carvalho et al., 2015), making it necessary to solubilize the xylan before hydrolysis to obtain high yields (Farias, de Araújo, Neri-Numa, & Pastore, 2019). As with any process, several factors influence the efficiency of enzymatic hydrolysis, including the structure of the lignocellulosic substrate, enzyme/substrate interactions, and the diverse composition of lignocellulosic biomass (Michel Brienzo, Carvalho, Figueiredo, & de Oliva Neto, 2016).

The complex structures that make up the xylan molecule are interconnected by covalent and ionic bonds, in addition to the presence of various branches, which is a limitation for the action of some enzymes. Therefore, a set of enzymes is needed to act on these branches and internal bonds of the molecule (Moreira & Filho, 2016; Sant'Anna, Souza, & Brienzo, 2014). Concerning the interaction between enzymes and substrates, hemicellulose hydrolysis occurs through endo enzymes that act internally in the main chain and exoenzymes that cleave oligosaccharides into monosaccharides. The endo-β-1,4-D-xylanase (EC 3.2.1.8) acts internally on the xylan backbone, randomly cleaving β-1,4-glycosidic bonds and generating shorter XOS (Beg, Kapoor, Mahajan, & Hoondal, 2001). These oligomers are subsequently hydrolyzed by exo-β-1,4-xylanase (EC 3.2.1.37), which cleaves XOS from the non-reducing ends, ultimately producing D-xylose. The synergistic action of these two enzymes enhances the hydrolysis process by accelerating the breakdown of xylan into xylose. For XOS production, an isolated/purified endo-β-1,4-D-xylanase is preferred, avoiding purification steps like removing xylose from the hydrolysate (Michel Brienzo et al., 2016). Furthermore, auxiliary enzymes are critical in enhancing XOS yield by removing specific side chains from the xylan backbone. These include the following: α-L-arabinofuranosidase (EC 3.2.1.55), which hydrolyzes arabinose substitutions (Juhász, Szengyel, Réczey, Siika-Aho, & Viikari,

2005); α-glucuronidase (EC 3.2.1.131), which cleaves glucuronic acid branches; and acetylxylan esterase (EC 3.1.1.72), which removes acetyl groups. By eliminating these structural hindrances, auxiliary enzymes increase the accessibility of xylanases to the main xylan chain, thereby improving the overall efficiency of enzymatic hydrolysis and increasing XOS yield (Moreira & Filho, 2016).

Given the advantages of enzymatic hydrolysis of xylan into XOS, studies have been conducted using enzyme cocktails, enzyme immobilization, and genetic modification of xylanases (Amorim, Silvério, Prather, & Rodrigues, 2019). Table 4 shows some studies using a combination of pretreatments and enzymatic hydrolysis utilizing xylanases from different sources and lignocellulosic substrates to produce XOS, such as *Bacillus subtilis* KCX006 using sugarcane bagasse as a source of xylan, as conducted by Reddy & Krishnan (Shyam Sunder Reddy & Krishnan, 2016a); wheat straw and *Bacillus halodurans* S7 (Faryar et al., 2015) and *Bacillus aerophilus* KGJ2 and corn cobs (Gowdhaman & Ponnusami, 2015). The highest XOS yield (99.6 %) was observed in these studies with xylanases from *Bacillus subtilis* KCX006 after 30 h, followed by the highest yields achieved with xylanases from *Bacillus halodurans* S7 (36 %) after 7 h and *Bacillus aerophilus* KGJ2 (11.4 %) after 12 h of hydrolysis.

Table 4 shows that the combined application of xylanases and auxiliary enzymes, such as arabinofuranosidase and feruloyl esterase, provides high yields and more fermentable XOS, as presented in the studies by Ávila et al. (Ávila, Franco Cairo, Damasio, Forte, & Goldbeck, 2020) and Pereira et al. (G. F. Pereira, de Bastiani, Gabardo, Squina, & Ayub, 2018). Ávila et al. applied a mixture of commercial enzyme cocktails in a defined proportion to produce XOS from sugarcane bagasse (Ávila et al., 2020). Among the xylan hydrolysis enzymes, β-xylosidase (EC 3.2.1.37) specifically catalyzes the hydrolysis of xylobiose and attacks the non-reducing ends of short XOS (de Freitas et al., 2019). It has recently been reported that exooligoxylanase (EC. 3.2.1.156) releases xylose from the reducing end of the xylan structure (de Freitas et al., 2019). Therefore, it is suggested that the activity of β-xylosidase should be minimal to reduce the release of xylose in the final product. Short XOS producing xylanase should be preferred as GH10 xylanases specifically produce short XOS compared to GH11 xylanases (Vishal Kumar, Bahuguna, Ramalingam, & Kim, 2021).

Immobilization techniques for xylanases have been widely used to reduce the costs of obtaining enzymes. The advantages of using immobilized xylanases are the longer cyclical use of enzymes in industrial-scale bioreactors, increased selectivity, stability, inhibition, and activity (Vishal Kumar et al., 2021). Xylanases have been immobilized on different support materials and applied to XOS production using different lignocellulosic agro-industrial residues (B. Wu et al., 2019). As shown in Table 4, the study by Wu et al. immobilized end xylanase from *Bacillus subtilis* using corn cobs as a substrate (B. Wu et al., 2019). This study found that the immobilization yield was close to 100 %, with a recovered activity of 82.4 %. The yield of XOS by the immobilized enzyme reached up to 515 mg/g of xylan, which was higher than that of the free enzyme, and the immobilized fusion enzyme was reused in five batches with XOS yields above 70 %.

More recently, Kim et al. investigated XOS production from bean stalks using endo-β-1,4-xylanase at 50 °C for 2 h, obtaining a yield of 105 mg/g. This result highlights the efficiency of enzymatic hydrolysis, suggesting bean stalks as a viable XOS source (I. J. Kim, Kim, Kim, Bornscheuer, & Nam, 2023). Dukare et al. studied the enzymatic hydrolysis of cotton husks under similar conditions (50 °C/2 h) using the same enzyme but achieved a lower yield of 45 mg/g (Dukare, Sharma, Nadanathangam, Nehete, & Saxena, 2024). This difference may be attributed to the biomass structure, which could reduce enzyme accessibility.

Lymperatou et al. investigated the hydrolysis of wheat straw using endo-β-1,4-xylanase at 50 °C, pH 4.8, 150 rpm for 48 h, obtaining a yield of 7.10 mg/mL (Lymperatou, Engelsen, Skiadas, & Gavala, 2023). This yield was lower than that reported by Kaur et al., possibly due to suboptimal reaction time and conditions for efficient XOS release from wheat straw (Kaur, Kaur, Kaur, Singla, & Taggar, 2024). In addition, the enzymatic hydrolysis of cotton husks (Dukare et al., 2024) and wheat straw (Lymperatou et al., 2023) resulted in lower yields, suggesting potential limitations in enzyme accessibility to the substrate.

On the other hand, Shao et al. investigated the use of fusion enzymes for XOS production, performing enzymatic hydrolysis at 40 °C, 200 rpm, for 72 h, obtaining a yield of 6.91 mg/g of biomass (Shao et al., 2024). This result suggests that fusion enzymes may be less effective than conventional xylanases used in other studies. In fact, the use of fusion enzymes (Shao et al., 2024) showed lower performance compared to conventional xylanases, indicating that this approach still requires improvements to enhance efficiency.

3.1.5. Unconventional approaches to XOS production

Xylan is organized in the xylan-lignin matrix, requiring pretreatment for extraction and subsequent enzymatic hydrolysis. However, economic feasibility is often hindered by low pretreatment yields (Amorim, Silvério, Prather, & Rodrigues, 2019; Mussatto & Teixeira, 2010). Various lignocellulosic sources have been studied, including integrated techniques within the biorefinery framework, emphasizing the need for sustainable processes with lower costs and higher XOS yields. New approaches, such as direct fermentation, solid-state fermentation, and deep eutectic solvents have being explored.

The microorganisms used for XOS production have the potential to reduce the amount of unwanted monosaccharides without additional purification steps. Studies by Amorim et al. (Amorim et al., 2018; Amorim, Silvério, & Rodrigues, 2019) integrated direct fermentation of untreated malt bagasse using xylanase-producing microbial strains from recombinant *Bacillus subtilis* and *Trichoderma* species, achieving promising yields: 463.4 mg XOS/g with *Bacillus subtilis* 3610; and 362.2 mg XOS/g with *Trichoderma reesei*. Direct fermentation offers advantages over commercial xylanases, reducing production costs without enzyme procurement or production. Gautério et al. (Victoria Gautério et al., 2018) also applied this technique using *Aureobasidium pullulans* OCCT, obtaining a XOS yield of 168 mg/g of xylan after 12 h of fermentation. However, process viability depends on waste composition, microorganism selection, and metabolite generation, with efficient lignocellulosic biomass degradation observed only with high xylan/lignin content waste and suitable microorganisms. Microbial fermentation yields essential XOS components, such as xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentose (X5), and xylohexose (X6), crucial for food and pharmaceutical applications due to their prebiotic effects.

As observed in the study by Reddy & Krishnan, solid-state fermentation is a new approach evaluated for xylanase and XOS co-production using *Bacillus subtilis* KCX006 from sugarcane bagasse (S. S. Reddy & Krishnan, 2016b). However, low yields of XOS (24.92 mg/g) were obtained under the optimal conditions, and the possible explanation is the need for specific process conditions during fermentation, which include optimal times other than those for optimizing XOS production (Amorim, Silvério, & Rodrigues, 2019).

Moreover, da Silva Menezes et al. used genetic recombination of xylanase-producing microorganisms and solid-state fermentation (da Silva Menezes, Rossi, Squina, & Ayub, 2018). A comparison was made between recombinant xylanase from *Aspergillus nidulans* XynC A773 and wild xylanase from *Aspergillus brasiliensis* BLf1 from rice husks in solid-state fermentation. There was a higher yield of XOS (75.92 mg/g of substrate) with *Aspergillus nidulans* XynC A773 than with *Aspergillus brasiliensis* BLf1 (37.25 mg/g of substrate), thus demonstrating that XOS yield can be higher when using recombinant xylanase rather than wild type xylanase.

Deep eutectic solvents (DES) are an emerging class of green solvents that have gained significant attention for their applications in biomass processing, particularly for XOS production (Loow et al., 2018). Comprising mixtures of hydrogen bond donors and acceptors, these solvents, when combined in specific proportions, create a liquid with

unique properties (Abbott, Capper, Davies, Rasheed, & Tambyrajah, 2002). DES exhibit favorable physicochemical properties, such as low toxicity and volatility biodegradability, and biocompatibility high thermal stability (Zdanowicz, Wilpiszewska, & Spychaj, 2018). These characteristics make DES an attractive and sustainable alternative to conventional solvents. In XOS production, DES are primarily used for the pretreatment of lignocellulosic biomass, facilitating hemicellulose hydrolysis into oligomers. Specific combinations, such as choline chloride and lactic acid, have demonstrated high efficiency in converting hemicellulose to functional XOS with a degree of polymerization below 5 (N. Kumar, Muley, Boldor, Coty, & Lynam, 2019; Li, Ding, & Zhao, 2019). Integrating DES with physical pretreatment methods, such as microwave irradiation, can significantly reduce processing times; overcoming one of the primary limitations of DES-based processes (Li et al., 2019). Additionally, DES have shown synergistic effects when combined with other pretreatment methods. Sequential hydrothermal and DES treatments have been effective in maximizing XOS yields and improving enzymatic digestibility, as demonstrated in studies using poplar sawdust (Santibáñez et al., 2021). DES can also be integrated with inorganic salts to enhance xylose recovery, as seen in the combination of choline chloride: urea with CuCl2 for oil palm fronds, further underscoring their versatility (Loow et al., 2018). Despite their potential, challenges such as the relatively long processing times and the need for optimization in industrial settings remain. However, DES offer notable environmental and economic advantages due to their green profile and ability to improve process efficiency (H. Xu et al., 2020). Their capacity to selectively solubilize polysaccharides, delignify biomass, and enhance sugar yields positions them as a promising tool for sustainable and scalable XOS production in biorefinery applications (Bjelić, Hočevar, Grilc, Novak, & Likozar, 2022; H. Xu et al., 2020). These methods enable high recovery rates of xylan, which is then converted into XOS, often achieving yields greater than 60 % (P. Kumar et al., 2024). By combining these treatment technologies, the ionic characteristic of the DES is maximized as microwave irradiation increases its polarity, facilitating the use of lower powers and shorter process times (Tan, Chua, & Ngoh, 2020). The study by Isci et al. (Isci et al., 2021) investigated microwave-assisted DES applications for xylan solubilization and XOS release from wheat straw. A 32 % yield of XOS with a DP below six was obtained, which comprised 43 % of the total XOS. Liu et al. (Xiao Liu, Wei, & Wu, 2019) performed a new pre-treatment with the synergism of lactic acid with DES, specifically the choline chloride/Lac system, on corn straw. This method made it possible to selectively obtain XOS with a degree of polymerization lower than five, representing 35 % of the product obtained. In addition, glucose was released in the proportion of 33.2 g per 100 g of material treated, and lignin was separated efficiently, with a yield of 40.9 % throughout the process. This innovative approach allows XOS production, fermentable sugar co-production, and the effective separation and valorization of the lignin present in the treated material. In the study by Shen et al. (Shen et al., 2021), a sequential hydrothermal pre-treatment with DES was conducted on poplar sawdust, resulting in the XOS co-production. The results indicated that a single hydrothermal pre-treatment produced 53.2 % XOS (based on crude xylan); however, the enzymatic digestibility was considered low. On the other hand, treatment with DES alone achieved effective enzymatic digestibility but with low XOS yields. However, when combined treatments were used, a high selectivity in lignin removal and a high glucose yield was observed. These results suggest that using sequential treatments with deep eutectic solvents could be a promising approach for XOS production and fermentable sugars, enabling efficient separation of the components of the lignocellulosic material, as well as maximizing the desired yields.

4. Biological properties of XOS conferring health benefits

4.1. Historical perspective on prebiotics

The definition of a prebiotic has changed over time, beings initially defined by Gibson & Roberfroid (Glenn R. Gibson & Roberfroid, 1995) as a non-digestible food ingredient that has a beneficial effect on the host by selectively stimulating the growth and/or activity of a limited number of bacteria in the colon, improving the health of the host. The International Scientific Association for Probiotics and Prebiotics (ISAPP) proposed a consensus definition in 2016 with a panel of experts in microbiology, nutrition, and clinical research. Since then, prebiotics have been defined as a "substrate used selectively by host microorganisms providing health benefits" (Glenn R. Gibson et al., 2017), and a wide range of microbial genera or species can consume a prebiotic substrate, excluding the restriction with selectivity and specificity related to the traditionally studied bacteria of the genus *Bifidobacterium* spp. and *Lactobacillus* spp. (Corim Marim & Gabardo, 2021).

To be classified as a prebiotic, a substance must selectively stimulate the growth or activity of specific microorganisms in the host (Glenn R. Gibson et al., 2017). The current definition extends prebiotic applications to the gastrointestinal tract, including extra-intestinal sites such as the skin and vagina, thus expanding its relevance to fields such as cosmetics (Amorim, Silvério, Prather, & Rodrigues, 2019) and animal health (poultry, cattle, and aquaculture) (Farias et al., 2019; Glenn R. Gibson et al., 2017). Dietary supplementation with XOS in poultry diets improves intestinal morphology, nutrient absorption, immune function, and gut health. These benefits are achieved by increased villus height, modulation of gut microbiota, and enhanced antioxidant and immune responses, making XOS a valuable additive for promoting poultry health and performance (Ding et al., 2018; X. Li, Wu, Ma, Chen, & Zhao, 2022; J. Zhou et al., 2021). For example, supplementing broilers with 75–100 mg/kg of XOS in a conventional corn-soy diet reduces drip loss in the thigh muscle, improves meat quality, and fosters intestinal health by reducing the depth of duodenal crypts (Suo et al., 2015). XOS enhances growth performance, feed efficiency, and gut microbiota balance in broilers, increasing beneficial bacteria such as Lactobacillus and Bifidobacterium while reducing harmful pathogens (Rao et al., 2024). It also boosts immune function by raising immunoglobulin levels and reducing inflammation. Using Xylo-Oligosaccharides (XOs) in animal nutrition is a promising market, particularly due to their gastrointestinal health benefits, applied in formulations for livestock, poultry, horses, and pigs (Valladares-Diestra et al., 2023). Research on XOs in animal health has focused on growth performance, nutrient digestibility, intestinal morphology, immune activity, antioxidant effects, and gut microbiota modulation (Y. Chen et al., 2021). Xylooligosaccharides (XOS) are prebiotics widely used in aquaculture due to their beneficial effects on fish health and performance. Research has shown that XOS enhance growth, feed conversion, and gut microbiota in Nile tilapia (*Oreochromis niloticus*) (Van Doan et al., 2018). Additionally, XOS support intestinal structural integrity by increasing the villus height/crypt depth ratio (Hahor, Thongprajukaew, & Suanyuk, 2019). Moreover, XOS supplementation has been found to improve growth performance and prevent intestinal apoptosis in grass carp (C.-Y. Sun et al., 2021).

In addition to traditional prebiotics, emerging candidates such as linoleic acid, polyunsaturated fatty acids, phytochemicals, and phenolics are also being explored for their potential in various applications (Glenn R. Gibson et al., 2017).

4.2. XOS as prebiotics

XOS are prebiotic oligosaccharides that help with gastrointestinal health as they resist digestion and reach the large intestine intact, due to the absence of enzymes capable of hydrolyzing β bonds. Thus, they selectively stimulate the growth of probiotic bacteria of the *Bifidobacterium* and *Lactobacillus* genera in xylose, xylobiose, and xylotetraose than in other carbon sources (Ayyappan Appukuttan Aachary & Prapulla, 2011; Amorim, Silvério, Prather, & Rodrigues, 2019). XOS has been shown to promote the growth of a limited number of microorganisms, such as *Bifidobacterium lactis*, *B. adolescent*, and *Lactobacillus brevis* (Ayyappan Appukuttan Aachary & Prapulla, 2011; Amorim,

Silvério, Prather, & Rodrigues, 2019; Crittenden et al., 2002; Moura et al., 2007). This specificity has not previously been observed with other prebiotics in pure cultures. Therefore, this property of XOS is advantageous, as it stimulates the growth of *Bifidobacterium* spp. and *Lactobacilli* spp. in the large intestine, suppressing bacteria that are harmful to health. It also reduces the concentration of harmful substances produced by fermentation in the gastrointestinal tract (Samanta et al., 2015). Furthermore, an important requirement to be considered an efficient prebiotic is the absence of gastrointestinal side effects (G. R. Gibson, Probert, Loo, Rastall, & Roberfroid, 2004).

4.3. Health benefits of XOS

These prebiotics are used and metabolized by probiotic bacteria, generating an increase in the production levels of short-chain fatty acids (SCFAs) such as acetate, propionate, butyrate, and lactate in the lower gastrointestinal tract. These SCFAs contribute to pH decrease (L. Liu, Li, Yang, & Guo, 2021; Zhang et al., 2020) and inhibiting or delaying the growth of pathogens such as *Clostridium* spp., *E. coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* (Ayyappan Appukuttan Aachary & Prapulla, 2011; Amorim, Silvério, Prather, & Rodrigues, 2019), which can be related to prevention of gut infection, suppression of colon cancer initiation, and improvement of intestinal health (Visekruna & Luu, 2021).

Thus, while the recommended daily dose of XOS that causes a significant effect on the proliferation of bifidobacteria is 1.4 g to 2.8 g/day, with no adverse gastrointestinal effects (Finegold et al., 2014), the recommended daily dose for fructooligosaccharides (FOS) is higher (10 g to 20 g/day) and may cause increased flatulence, intestinal distension and irritation (Macedo, Vimercati, & Araújo, 2020). These differences highlight the specificity and advantages of XOS over FOS and other established prebiotics as effective and well-tolerated prebiotics for the human body.

In addition, XOS consumption offers other health benefits, as they have been linked to a reduction in the formation of harmful compounds, such as secondary bile acids, which are known to have carcinogenic properties (Glenn R. Gibson & Roberfroid, 1995; Moure et al., 2006) as well as a reduction in the risk of colorectal cancer, while improving symptoms in conditions such as Crohn's disease and ulcerative colitis (Ayyappan A. Aachary et al., 2015). Xylo-oligosaccharides (XOS) have shown the ability to lower glycated hemoglobin (HbA1c) and fructosamine levels, as well as improve lipid profiles by reducing total cholesterol, low-density lipoprotein (LDL), and apolipoprotein B, which transports cholesterol into cells. HbA1c reflects average blood glucose levels over the past 2–3 months and is commonly used to diagnose and manage diabetes, with higher levels indicating poor long-term glycemic control (ADA, 2020). Fructosamine, formed when glucose binds to proteins (primarily albumin), provides a short-term measure of glycemic control, typically reflecting blood glucose over the past 2–3 weeks (Nansseu et al., 2015). XOS can reduce these markers, improving blood sugar and lipid levels in individuals with obesity and type 2 diabetes mellitus (Sheu, Lee, Chen, & Chan, 2008). These benefits suggest that XOS may offer therapeutic potential for improving metabolic health.

Furthermore, XOS exhibits antioxidant properties due to hydroxycinnamic acids, such as ferulic acid, and coumaric acid in its xylan chain (Ayyappan Appukuttan Aachary & Prapulla, 2009) stimulates the immune system for a more effective immune response (Zhenping et al., 2013). It also demonstrates anti-inflammatory and antimicrobial activity, helping to reduce inflammation and combating unwanted microorganisms (Hansen et al., 2013). These characteristics make XOS promising compounds with potential nutraceutical and functional applications. However, it is crucial to acknowledge that the efficacy of these properties can vary depending on concentration, form of administration, and the biological context in which they are used. In other words, although XOS consumption seems promising, caution should be exercised in interpreting its potential health benefits until more scientific data are available.

5. XOS applications

XOS are commercially used mainly in animal feed, pharmaceutical/medical products, and cosmetics, however, food applications have gained greater prominence due to their favorable chemical and biological properties for this type of application (EFSA, 2018; Palaniappan et al., 2021). XOS are used in animal feed as developing growth stimulants, and as a full or partial replacement for antibiotics (M. Gupta & Bajaj, 2017; Vázquez et al., 2000). In the study by Abasubong et al. XOS were supplemented with a rice protein concentrate to replace wheat flour in fish feed, and positive results were observed regarding intestinal enzyme activity, intestinal morphology, and amino acid metabolism (Abasubong et al., 2019). Wang et al. confirmed the potential of XOS and polysaccharides from a medicinal plant (*Astragalus* spp.) with gamma radiation on the growth performance and barrier function of the intestinal mucosa of broiler chickens (Q. Wang et al., 2021). The results indicated that this combination acted as a potential substitute for the antibiotic chlortetracycline, improving growth development, intestinal morphology, and the intestinal barrier in broilers.

Wang et al. evaluated the effects of dietary XOS supplementation in diets low in fishmeal on triploid rainbow trout, the main species of trout farmed in cold water in China (C. Wang et al., 2022). The results showed that XOS promoted this trout species' growth and intestinal health, stimulating proper intestinal development, reducing damage and inflammation, and regulating the intestinal microbiota. Sun et al. (C.-Y. Sun et al., 2021), supplemented the diet of grass carp (*Ctenopharyngodon idella*) with XOS, growing for 60 days. The authors confirmed for the first time that adequate levels of XOS supplementation improved grass carp growth, increased the number of *Lactobacillus* and *Bifidobacterium* and the concentration of SCFAs, and prevented intestinal cell apoptosis. Previously, Zhang et al. (Zhang et al., 2020b) experimented to evaluate the effects of XOS on the growth, immunity, and disease resistance of juvenile grass carp (*Ctenopharyngodon idella*). The results showed that the plasma content of triglycerides was significantly lower in fish fed on a diet containing 0.05 % and 0.1 % XOS compared to the control group.

Other interesting properties, such as antioxidant and moisturizing capacity, have stimulated the XOS application in the cosmetics industry (Moure et al., 2006; Valls et al., 2018), for example by the company MySkinRecipes, which already uses 70 % pure XOS as a cosmetic ingredient. However, studies on cosmetic applications have been under explored. Considering the recent update of the prebiotic concept, including action at extra-intestinal sites, there is expected to be an increase in interest in applying XOS in cosmetic products (Amorim, Silvério, Prather, & Rodrigues, 2019). The precursor study by Chang et al. (Chang et al., 2020) used red fruit juice with XOS to evaluate the benefits on skin health. There is little clinical evidence for applying XOS in anti-aging. Improvements in radiance, moisture, elasticity, and reduction of skin blemishes were observed. These results indicate that prebiotic juice may offer an alternative route for daily skin care. However, the exact mechanism by which XOS improves the skin is not fully understood.

Another important application of XOS has been developing more sustainable packaging. From xylan and high molecular mass XOS, ethers and esters have been produced and used as thermoplastics for biodegradable plastics, water-soluble films, edible food coatings (Corim Marim & Gabardo, 2021), as well as chitosan xylan capsules and hydrogels, whose main advantage is that they cause less environmental impact due to their shorter biodegradation time (Moure et al., 2006).

6. Physicochemical, technological, and sensory properties of XOS for food applications

Xylooligosaccharides (XOS) obtained from lignocellulosic biomass have interesting physicochemical, technological, and sensory properties

for industrial food applications (Fig. 3) They have significant solubility in water (Vázquez et al., 2000); highly hygroscopicity (Alonso, Domínguez, Garrote, Parajó, & Vázquez, 2003); high pH stability (between 2.5 and 8); resistance to high temperatures (up to 100 °C) (Moure et al., 2006), and are used for example in low pH juices, carbonated drinks, acidic foods, and pasteurized products (M. Gupta & Bajaj, 2017).

Although the emulsifying capacity of XOS is underexplored, ongoing research aims to investigate this property (Paschoa et al., 2024) evaluating the replacement of pork fat in sausages with an XOS-rich gel emulsion, demonstrating that it is a viable alternative, preserving similar physicochemical and technological properties to the original products. Moreover, it contributes to developing healthier meat products with prebiotic properties, reduced fat content, and lower energy value. Despite its limited application in meat technology, XOS shows great potential in gel emulsions to replace saturated fat in meat products due to its excellent technological properties, such as high water retention, broad pH range, and thermal stability (Ávila et al., 2020; Palaniappan et al., 2021; Paschoa et al., 2024).

Another technological property of XOS is their ability to interact with water molecules, which results in greater moisture retention (Moure et al., 2006); it is particularly useful in bakery products, improving texture, preventing dryness, and extending the shelf life of these products (Palaniappan et al., 2021).

In addition, XOS have acceptable sensory characteristics such as mouthfeel, flavor, texture, and color (Hirayama, 2002); sweetness equivalent to 30 % of sucrose (Samanta et al., 2015); anti-cariogenic property (used for xylitol production) and sweetening power making them ideal ingredients for functional foods and dietary sweeteners for diabetic patients (Finegold et al., 2014; Vázquez et al., 2000). The recommended daily dose is 1.4 to 2.8 g/day for healthy adults (Finegold et al., 2014) for different age groups (≤11 months to ≥65 years) (Palaniappan et al., 2021). Commercially available XOS syrup demonstrates a sweetness capacity equivalent to 50 % sucrose, accompanied by physicochemical stability during processing conditions. This offers promising opportunities for their use as alternative sweeteners in food products (Kyung et al., 2014). This characteristic of XOS may make it possible to reduce the amount of sucrose used in foods and drinks to reduce sugar consumption and the associated health impacts.

XOS has cryoprotective properties, acting as an antifreeze agents and capable of protecting food from damage during freezing (Moure et al., 2006), as observed in meat products such as meatballs (Y.-B. Wu & Lin, 2011). In addition, they can increase the fiber content in foods, such as baked goods, providing additional nutritional benefits (Palaniappan et al., 2021). These properties make XOS versatile and functional ingredients in the food industry, making it possible to improve the quality, stability, and nutritional composition of a variety of food products.

Another promising application of XOS is to improve the consistency of gels and preserve compounds such as vitamins or probiotics. The study by Martins et al. (Martins, Silva, Ávila, Sato, & Goldbeck, 2021) reported that alginate microparticles containing 3 % XOS increased the survival of encapsulated *Lactobacillus acidophilus* during refrigerated storage and simulated digestion by strengthening the gel and reducing the pore size of the capsule. Another study showed that XOS influenced the rheological properties and formed a gel network with high viscosity to protect an encapsulated vitamin complex based on pectin and lentil protein (Mekala, Silva, & Saldaña, 2022). Although the preliminary results are promising, further research is needed to fully explore the mechanisms involved in this application to determine the feasibility and real effectiveness of using XOS to form hydrogels and preserve bioactive compounds.

XOS has many beneficial physicochemical and technological properties, but some shortcomings can limit their application in various types of food, such as water solubility. In addition, the addition of XOS can interfere with the sensory characteristics of foods, affecting their taste, aroma, and texture, which can be undesirable in certain products. Regulatory restrictions regarding using XOS in foods may also exist, varying according to the regulations in each location. In addition, obtaining XOS from lignocellulosic biomass can be complex and costly, limiting their commercial availability. It is therefore necessary to develop new studies related to technological approaches to overcome these shortcomings and allow a wider application of XOS in the food industry.

7. XOS as a food ingredient

Given the physicochemical, technological, and sensory properties of XOS, studies have been conducted to explore its potential as a new food ingredient in various food matrices, such as animal products, dairy products, baked goods, vegetable proteins, vegan products, and non-alcoholic carbonated drinks. However, most studies on XOS focus on

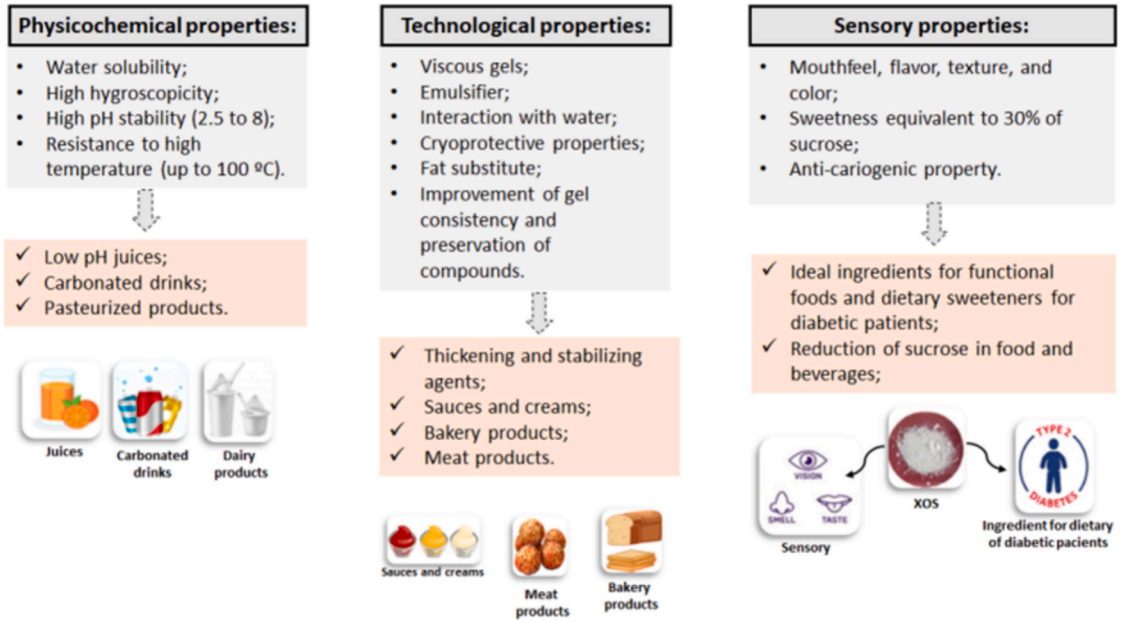


Fig. 3. Physicochemical, technological, and sensory properties of XOS for food applications.

developing more economical and sustainable production processes rather than investigating its various applications in the food industry. In addition, compared to other prebiotics such as FOS and inulin, there is a lack of research examining incorporating XOS into foods and drinks. It is, therefore, crucial to expand research into the fields of application of XOS to take full advantage of their potential as a versatile and functional food ingredient.

Regarding functional foods, manipulating the gut microbiota in a beneficial way is a strategy to manage or improve the health of the host and can be achieved using probiotics, prebiotics, and symbiotics (Swanson et al., 2020). In 2019, the International Scientific Association for Probiotics and Prebiotics (ISAPP) updated the definition of a symbiotic as "a mixture consisting of live microorganisms and substrate(s) selectively utilized by host microorganisms to confer health benefits to the host". This new definition also includes two approaches to formulating symbiotic: complementary and synergistic. A complementary symbiotic involves combining a probiotic with a prebiotic (or more than one of each), which acts independently to achieve one or more health benefits. On the other hand, a synergistic symbiotic comprises a living microorganism and a substrate designed to work together, where the substrate is selectively used by the co-administered microorganism (Swanson et al., 2020).

XOS can also be used as an ingredient in developing and/or reformulating synbiotic products, where the interaction between probiotic and prebiotic is favored in the host organism (Swanson et al., 2020). Childs et al. (Childs et al., 2014) conducted a study with healthy volunteers using *Bifidobacterium animalis* subsp. *lactis* Bi-07 (109 CFU per day) and 8 g per day of XOS. In this research, XOS increased the count of bifidobacteria in the feces of both the groups receiving the combined product and the groups that received only prebiotics, compared to the control group. Additionally, the study observed improvements in plasma lipid profiles and modulated markers of immune function in healthy adults. This study complies with the new definition of a complementary synbiotic. However, an increase in bifidobacteria was not observed in the individuals who consumed the combined product, thus not meeting the new definition of a synergistic synbiotic.

An example of a commercially available symbiotic product is the *Bikkle* product, manufactured by Suntory Ltd. in Japan since 1993. This drink consists of XOS, whey minerals, oolong tea extract, and bifidobacteria (M. Gupta & Bajaj, 2017). This new definition is intended to encourage innovation in formulations, allowing components not to be limited to rigid definitions of probiotics or prebiotics (Swanson et al., 2020). Therefore, this more flexible approach is intended to stimulate the development of new synbiotic products in the food industry, enlarge the options available to promote intestinal health, and improve consumer health.

7.1. Applications in products from animal sources

Early research revealed the freeze-protective effect of a combination of xylose-derived, such as xylobiose and xylotriose, on during freezing (Chou & Lin, 2010). Gel strength increased proportionally to XOS concentration up to 6 %, while protein solubility improved as XOS concentration increased to 14 %. Compared to other oligosaccharides, XOS showed a lower occurrence of syneresis (Martins et al., 2023). This cryoprotective effect on animal products has been evaluated in many studies, from the physicochemical and sensory properties of Chinese meatballs (Y.-B. Wu & Lin, 2011); the stability of meat patties during freezing (Y.-B. Wu & Lin, 2014); cryoprotective properties and the interactions of XOS with shrimp muscle proteins (Zhang et al., 2018). Shrimp soaked in XOS solutions (3.0 % *w/v*) were found to have better water retention capacity, myofibrillar protein stabilization, and texture properties than the other treatments.

7.2. Dairy products

XOS have been used in a variety of dairy products, including yogurts (Li, Ding, & Zhao, 2019; Penksza, Juhász, Szabó-Nótin, & Sipos, 2020), cottage cheese (Ferrão et al., 2018), strawberry flavored milk drinks (Souza et al., 2019), and "dulce de leche" (Leddomado et al., 2021).

A pioneering study revealed the functional properties of a strawberry-flavored milk drink formulated with XOS (Souza et al., 2019). This product demonstrated a significant increase in important functional properties, such as inhibiting enzymes related to controlling hypertension and diabetes in in vitro tests. In addition, the rheological characteristics of the drink were modified, resulting in greater viscosity as the XOS was incorporated into the formulation. In addition to the functional properties, the product also showed an improved volatile profile and good sensory acceptance, ensuring adequate concentrations of the prebiotic (1.25 g/mL) (Souza et al., 2019).

Research shows that whey-based beverages enriched with XOS exhibit improved functional characteristics and additional effects, such as enhanced antioxidant activity and inhibition of angiotensin-converting enzyme (ACE), α-amylase, and α-glucosidase (Ribeiro et al., 2021). It is already known that, ACE inhibition is associated with reduced blood pressure, contributing to its anti-hypertensive effects (Silveira et al., 2019) and this approach has also been validated in clinical studies as an effective method for managing hypertension, a major risk factor for cardiovascular diseases (Whelton et al., 2018). Additionally, ACE inhibition is linked to anti-diabetic effects through the inhibition of α-amylase and α-glucosidase (da Silva et al., 2020). Bioactive compounds in cold plasma-treated beverages, particularly those treated for longer durations, may enhance nutritional quality, showing improved antioxidant, anti-hypertensive, and anti-diabetic properties. These findings suggest the potential of such beverages in managing hypertension and diabetes, although further clinical trials are needed to confirm the clinical significance of ACE inhibition in this context.

XOS were added to curd cheese as a fat substitute and prebiotic agent, resulting in better physicochemical and rheological characteristics (Ferrão et al., 2018). The viscosity increased and greater melting resistance were attributed to particle size and water absorption capacity. The authors concluded that XOS could be a potential fat substitute with the advantage of obtaining a product with characteristics similar to a full-fat product. Moreover, in studies on cream cheese and whey drinks, the same concentration of XOS was used in the formulations, aiming to achieve a daily dose of 1.4 g (Finegold et al., 2014).

Recently, XOS were applied together with inulin in "dulce de leche", and a firmer, gummy texture was observed, regardless of the fat content, providing better functionality for the product (Leddomado et al., 2021).

7.3. Bakery products

XOS have been used in a variety of bakery products, including cookies (Ayyappan et al., 2015; Pareyt, Finnie, Putseys, & Delcour, 2011), *Idli* - a fermented cereal based-cake (Ayyappan Appukuttan Aachary & Prapulla, 2011), rice cake (C. Yang et al., 2021), and bread (Palaniappan, Yuvaraj, Sonaimuthu, & Antony, 2017).

Pareyt et al. (Pareyt et al., 2011) observed that adding XOS to cookies starting at 30 % resulted in diameter and height comparable to the control but with a slightly darker color. Additionally, Ayyappan et al. (Ayyappan et al., 2015) found that after 21 days of storage, the cookies with 5 % XOS were highly acceptable in terms of physicochemical properties, maintaining a similar moisture to the control, while higher concentrations of XOS significantly affected moisture, which had an impact on the final texture. Regarding bread and the incorporation of XOS, Ayyappan & Antony (Palaniappan et al., 2017) observed that adding 30 % XOS to the dough resulted in stable dough and a better bread volume.

In the study by Aachary & Prapulla (Ayyappan Appukuttan Aachary

& Prapulla, 2011), the addition of 0.4 % XOS to a fermented cereal-based cake known as *Idli* resulted in a longer fermentation time of 6 h, resulting in adequate texture, color, and sensory characteristics, as well as a higher moisture content and softer texture. A more recent study by Yang et al. (C. Yang et al., 2021) observed that adding XOS to rice cakes significantly reduced the loss during cooking. Furthermore, this addition promoted the regeneration of starch, resulting in a decrease in the digestibility rate.

7.4. Other food applications

Plant proteins often have low solubility, which limits their application. However, it has been observed that the combined use of XOS and plant proteins can improve their functionality. The combination of XOS and pea protein improved thermal stability, antioxidant activity, and solubility (Zhao et al., 2022). These promising results provide a basis for developing new plant-based foods with the potential to offer functional benefits.

The application of XOS in vegan products has aroused interest due to their benefits. XOS can be used as functional ingredients in substitutes for animal products, such as vegan cheeses, yogurts, and desserts, providing desirable technological and sensory properties. In addition, these compounds can act as prebiotics, stimulating the growth of beneficial bacteria in the gut and promoting digestive health. Using XOS in vegan products expands the options available to consumers and contributes to creating more nutritious and sustainable foods.

Vegan products incorporating XOS can already be found commercially, such as sparkling fermented tea and Wonder Drink Kombucha (Martins et al., 2023). In addition, many other vegan products that include XOS as an ingredient are being developed. The use of XOS in vegan products offers additional benefits, such as prebiotic properties and improvements in the texture and taste of food. This trend reflects the growing demand for vegan options on the market and the recognition of the potential of XOS as functional ingredients in the food industry.

The study by Dai et al. (H. Dai, Leung, Corradini, Xiao, & Kinchla, 2020) found that the limited concentration of XOS in strawberry puree resulted in minimal quality changes (rheology, color, water activity, pH, and total soluble solids). Optimizing the concentration of XOS to 5 % (*w/w*) did not significantly alter the quality attributes of the strawberry puree. This proposal for supplementing strawberry puree could be a viable solution for increasing consumers' fiber intake.

In addition, XOS showed a positive impact when added to a non-alcoholic carbonated drink with an intense sweetener (a mixture of acesulfame K and aspartame). It was observed that by adding XOS, the full-bodied character of the drink was significantly enhanced without any disadvantage in the perception of unpleasant taste or mouthfeel (P. K. Gupta et al., 2012).

8. Market, economic and environmental factors

The global probiotic and prebiotic markets are experiencing significant growth, reflecting increasing consumer demand for gut health solutions. The probiotic industry reached a market value of $7.4 billion in 2023 and is projected to grow to $20.71 billion by 2030 (Gruening de Mattos et al., 2024). Similarly, the prebiotics market is expanding rapidly, with a valuation of $6.95 billion in 2023 and an expected compound annual growth rate (CAGR) of 11.37 % over the forecast period Research and Markets, 2024. This upward trend highlights the growing recognition of the health benefits associated with probiotics and prebiotics, driving their increasing integration into functional foods, nutraceuticals, and dietary supplements. FOS, inulin, lactulose, and galactooligosaccharides (GOS) are prebiotics already established in Europe, Japan, and the United States. Japan is the largest producer and consumer of prebiotics and the pioneer in incorporating XOS into food and feed, and much of the scientific evidence on these emerging prebiotics has been conducted by Japanese and European researchers (Michel Brienzo, Carvalho, & Milagres, 2010; Vázquez et al., 2000).

The XOS market, which is expanding, is expected to increase by 130 million dollars in 2025 compared to 2023 (Valladares-Diestra et al., 2023). Asian countries are the largest XOS market, in which Japan had the largest production and consumption of XOS worldwide (de Freitas et al., 2019). The most important XOS industries in the world include: Longlive (Shandong, China), Yuan Long Chem (Tianjin, China), Henan Shengtai Biotechnology Co. (Zhengzhou, China), Henan Kangwei (Xinjiang, China), HFsugar (Xinjiang, China), and Yibin Yatai (Sichuan, China) (Martins et al., 2023).

It is important to note the projected growth between 2023 and 2029 for the segment of plant sources for producing functional ingredients such as prebiotics (Markets and Markets, 2024). Thus, as XOS can be produced from plant raw materials, such as lignocellulosic agro-industrial waste, it is hoped that this increase in the XOS market will encourage new research into new sources and methods of producing high yield XOS with suitable functionality and application. The market price of XOS ranges from US$25,000/ton to US$50,000/ton, depending on the purity of the powder or syrup (Han et al., 2020). In addition, they are considered the most competitively priced prebiotics in terms of the recommended daily dose to achieve the prebiotic effect (Finegold et al., 2014). Therefore, XOS competes for market share as its potential and multidimensional benefits are widely disseminated and proven (Amorim, Silvério, Prather, & Rodrigues, 2019).

Fig. 4 presents relevant information about the global panorama of prebiotics, including XOS, established prebiotics worldwide, and Japan's notable position as the market leader in this segment. Within the prebiotic category, the XOS market stands out, as it is expanding, focusing on its market price impact. Additionally, Fig. 3 highlights the co-production of XOS from lignocellulosic materials using the biorefinery concept, which provides economic and sustainable benefits.

An interesting XOS production alternative includes co-production in lignocellulosic material biorefineries, as observed also in Fig. 4. The XOS co-production in lignocellulosic biorefineries is a sustainable strategy that combines economic viability with environmental impact reduction, particularly in terms of carbon footprint and resource efficiency. Greenhouse gas (GHG) emissions in this process stem from extraction, hydrolysis, and purification, which require energy and chemical inputs. However, utilizing agro-industrial residues as feedstock significantly reduces these emissions by preventing improper disposal and minimizing the need for additional crops (Bhatia, Jha, Sarkar, & Sarangi, 2023; Vishal Kumar et al., 2021; Manicardi et al., 2023).

To mitigate the carbon footprint of XOS production, key strategies include optimizing enzymatic hydrolysis, using renewable energy sources, and reducing chemical reagents. Additionally, Life Cycle Assessment (LCA) studies are crucial for quantifying environmental impacts and optimizing processes to improve efficiency and sustainability. LCA serves as a tool to evaluate the environmental, energy, and material impacts associated with production (Prasad et al., 2020; Yin et al., 2024).

From an economic perspective, integrating XOS production with other bioproducts enhances biorefinery profitability by increasing financial returns and diversifying revenue streams. This approach maximizes lignocellulosic residue valorization, reduces dependence on a single product, and improves cost-effectiveness. Moreover, marketing XOS as a high-value prebiotic helps offset the high operational costs of second-generation technologies, which remain costly and technologically immature (Brandt et al., 2020).

Environmentally, XOS co-production aligns with circular economy principles by converting agricultural residues into valuable bioproducts and reducing emissions from waste decomposition. The comprehensive utilization of lignocellulosic biomass for multiple products further decreases reliance on fossil-based raw materials, mitigating GHG emissions (Kaushik & Kataria, 2024). Product diversification in biorefineries enhances bioethanol sustainability by improving biomass efficiency and minimizing large-scale biofuel production impacts (Mizik, 2021).

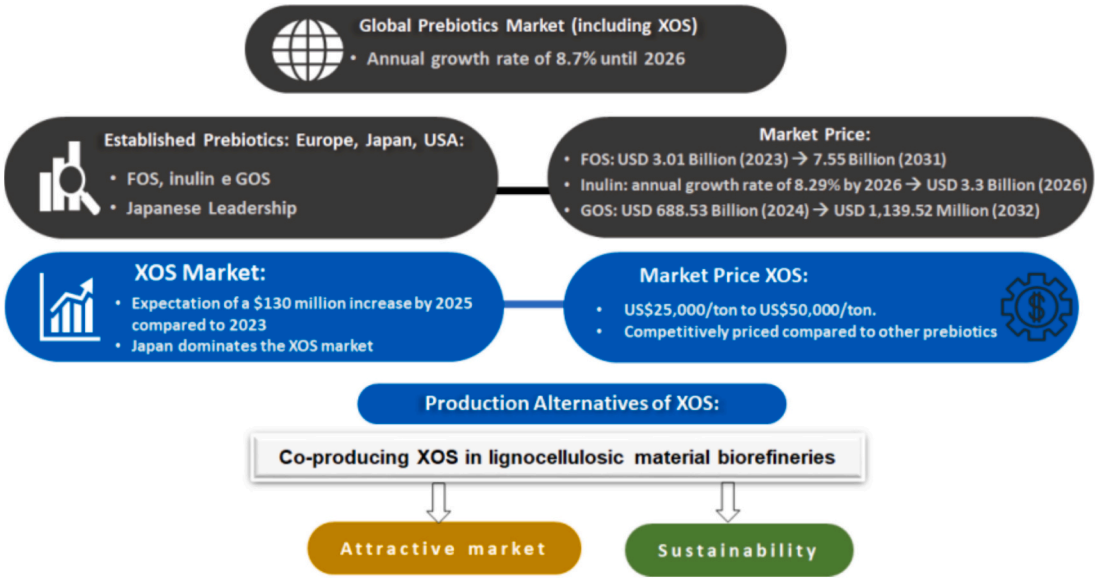


Fig. 4. Market aspects of prebiotics and XOS: production alternatives in biorefineries for economic attractiveness and sustainability (Bridge, 2025; Michel Brienzo et al., 2010; Gomes et al., 2021; Valladares-Diestra et al., 2023; Vázquez et al., 2000) (RESEARCHandMARKETS, 2024; Michel Brienzo et al., 2010; Vázquez et al., 2000, Valladares-Diestra et al., 2023; Han et al., 2020; Gomes et al., 2021; Bridge, 2025).

Efficient resource utilization is essential for sustainable XOS production, offering both economic and environmental benefits. The process requires water, energy, and chemicals for biomass conversion. Water reuse and recycling can reduce consumption, while recovering by-products and co-products optimizes resources. Using eco-friendly solvents and catalysts minimizes toxicity and waste, and valorizing non-XOS fractions for biofuels or biocompatible materials further reduces the environmental impact (Ali et al., 2025; Manicardi et al., 2023).

When obtaining XOS as an additional section in a biorefinery of eucalyptus residues with biofuel production, a significant increase in costs was observed after purchasing the equipment necessary for XOS purification, reporting a significant impact on capital investment and annual facility-dependent costs (Gomes, Michelin, Romaní, Domingues, & Teixeira, 2021). The results obtained, however, indicated notable additional revenue (121 % growth in total revenue) from the sale of the product. The authors demonstrated an increase in annual revenue of around $5 million (approx. R$19.75 million), reversing a negative net present value (NPV) of $-3.4 million to $18.9 million, even with an increase in operating costs. In this technical economic study, the authors considered the selling price of XOS at 50$/kg.

9. Regulatory aspects of XOS

The use of XOS in food is regulated in North America and Europe by the Food and Drug Administration (FDA, 2013) and the European Food Safety Authority (EFSA, 2018), both of which affirm the safety of oligosaccharides. However, in Brazil, XOS does not yet have specific legislation regulating their use (Amorim, Silvério, Prather, & Rodrigues, 2019). Among the XOS manufacturers assessed as GRAS (Generally Recognized as Safe) in 2013, corn cob XOS, manufactured by Shangdong Longlive Biotechnology (China), received this certification from tests conducted on humans when used as a food ingredient (FDA, 2013). In 2015, XOS, produced via enzymatic hydrolysis from corn cobs (Longlive), were evaluated by the EFSA Panel on Dietetic Products, Nutrition, and Allergies, requested by the European Commission, concluding that XOS did not present any toxicity for human consumption (EFSA, 2018). Sugarcane fiber XOS, manufactured by Prenexus Health (USA), has also been certified as GRAS for use as a texturizer and ingredient in various food categories.

XOS production has been registered since 1995. According to the *Derwent* database, there are currently 659 patents registered in different fields of application, and the main patents registered on XOS are from 2012 to 2018, most of which are of Chinese origin (Valladares-Diestra et al., 2023). In addition to the projected growth in market value for XOS, industrial interest in these compounds is also reflected in the increase in the number of patents for various types of applications, such as detox beauty gel (CN107397171B, 2018); food for weight loss (CN106107293B, 2017); nutritional formula for cancer patients (CN104814375B, 2018); odor reduction feed for pigs (BE1023808B1, 2017); microencapsulation (CN105831784B, 2018); constipation treatment (CN105053778A 2015; CN106361741B, 2017) (Amorim, Silvério, & Rodrigues, 2019).

Given the current growth phase of the XOS market, it is expected that new regulatory actions will be approved for each region in the world, as well as intense investment in research and innovation activities as there are few experimental studies on humans with XOS and AXOS, for example. Thus, by providing new scientific evidence about the prebiotic efficacy of these oligomers, it is hoped to obtain new health claim approvals, which will also favor the acceptance of these prebiotics by consumers (Amorim, Silvério, Prather, & Rodrigues, 2019; Benson et al., 2018).

9.1. Aspects of Brazilian legislation on XOS

In Brazil, the Brazilian Health Regulatory Agency (ANVISA) establishes the minimum quantities of prebiotic substances required for a product to make a functional food claim. These regulations fall under the category of “Foods with Functional and/or Health Claims,” where the health claim states, suggests, or implies a relationship between the food or ingredient and a disease or health-related condition. It is mandatory for foods that advertise this functional or health property claim to be registered with ANVISA, 2018 (https://www.gov.br/anvisa/en).

These regulations aim to ensure that functional and health claims are based on solid scientific evidence, providing consumers with reliable information about the benefits of food. It is important to note that ANVISA guidelines are updated periodically, therefore it is essential to consult the latest regulations to obtain up-to-date information on the specific requirements for functional and health property claims in Brazil.

Regarding Brazilian legislation for XOS, no specific legislation

currently regulates its use (Amorim, Silvério, Prather, & Rodrigues, 2019). However, four applications containing xylo-oligosaccharides in the product composition are currently under analysis or being processed, as presented in the Report on the Analysis of Contributions to CP No. 456/2017 (ANVISA, 2018). For the claim to be approved, a technical, scientific report needs to be submitted that includes clinical and/or epidemiological trials and a review of the scientific literature to prove the reported effect.

Food and supplement manufacturers and distributors containing xylo-oligosaccharides must comply with ANVISA regulations, ensuring safety and compliance with labeling standards. This includes obtaining authorization to use these additives and complying with appropriate labeling requirements, clearly stating the presence and quantity of XOS in the products.

10. Conclusions

This work addressed the production of oligosaccharides from lignocellulosic biomass, mainly XOS, including their functional properties, food applications and market insights. XOS are emerging prebiotics with diverse applications, particularly as food ingredients, exhibiting significant economic implications across various sectors. However, further in vivo experimental studies are required to comply with prevailing regulatory legislation in different countries, thereby expanding its market potential.

Moreover, XOS derived from readily available and cost-effective lignocellulosic raw materials, such as agricultural and agroindustrial waste, contribute substantially to the value chain of these agricultural sectors. On the other hand, the development of emerging production strategies, including fermentation and genetic engineering, are notable trends to develop super microorganisms capable of directly converting lignocellulosic biomasses into XOS. However, as most studies on XOS focus on developing more cost-effective and sustainable production processes, more efforts are needed to investigate more applications in the food industry.

CRediT authorship contribution statement

Solciaray C.S.E. de Paula: Writing – original draft. **Victor Haber Pérez:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Maria I.S.R. de Sousa:** Writing – original draft. **Djully L.G. Ribeiro:** Writing – original draft, Conceptualization. **Claudia Amorim:** Conceptualization, Writing – review & editing. **Marcelo H. Vasconcelos:** Writing – original draft. **Júlio C. dos Santos:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Michel Brienzo:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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Data availability

Data will be made available on request.

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