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Chapter 8

**SUGARCANE BAGASSE
HEMICELLULOSE PROPERTIES,
EXTRACTION TECHNOLOGIES AND
XYLOOLIGOSACCHARIDES PRODUCTION**

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ABSTRACT

The sugarcane has been used for centuries for sugar production and, in the recent decades, for ethanol fuel production through biotechnological routes. From sugar and ethanol industry a large amount

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of waste is generated as a by-product, the sugarcane bagasse (SCB). This waste is one of the largest lignocellulosic material (LCM) resources, characterized by its chemical composition based on the main components of cellulose, hemicellulose and lignin. SCB is burned for energy cogeneration; however, several applications have been studied based on its components, e.g., hemicellulose has been studied for the production of biofuel (xylose fermentation to ethanol), organic acids, artificial sugar (xylitol), and derivatives such as xylooligosaccharides (XOS). In spite of its several important applications, the hemicellulose extraction or solubilization from LCM is not simple. Hemicellulose is associated with cellulose and lignin in the plant cell wall in a highly organized structure. The LCM is recalcitrant and resistant to biodegradation or biotechnological conversion routes. The LCM requires a pretreatment step to change the accessibility of the polysaccharides or these need to be extracted for further uses. The hemicellulose can be extracted based on chemical (acidic or alkaline treatment) and enzymatic processes, which depends on the final application (mono, oligo or polysaccharides). XOS are special oligomers with potent prebiotic effect, usable as additives for food and feed. However, they are poorly exploited due to the cost and technological limitations. In this review were discussed the SCB hemicelluloses properties and the key technologies involved in the hemicellulose extraction and XOS production. Additionally, studies that prove the effectiveness of the use of these oligomers as a prebiotic for human and animal were presented from recent reports.

Keywords: sugarcane bagasse, hemicellulose, xylan, alkaline extraction, xylanases hydrolysis, acid hydrolysis, autohydrolysis, xylooligosaccharides, prebiotic effect

INTRODUCTION

The research of new alternatives for energy and food is a global concern due to technological, physical and environmental limitations. There has been an increase in agricultural waste production because of an intensive population growth and thus this knowledge is required for food and fuels. The problem begins with the inappropriate disposal of generated wastes that can provoke environmental problems. Furthermore, these agricultural wastes can be used as a feedstock material to biotechnological processes, in order to produce energy and added-value molecules. To use waste material so as to convert it into a valuable product a pretreatment or a process for biomolecule extraction is necessary. Despite several decades of study, there are still some questions that

need to be answered about LCM. These specific wastes from agriculture and forest are recalcitrant and resistant to enzyme action, characterized by their heterogeneity (Brienzo et al., 2014).

Sugarcane bagasse (SCB) is currently one of the most important lignocellulosic wastes generated from sugar and ethanol production. Brazil is the largest producer, responsible for 40% of the worldwide production. The sugarcane is cultivated in Brazil to produce sugar (since many centuries ago) and ethanol and electricity (in the last decades) in an integrated plant. The SCB is the waste generated after sugarcane is crashed for sucrose extraction. From the sugarcane harvesting another waste is generated: the straw, of which part is left on the field for soil fertilization. Both of the LCM, bagasse and straw, are materials rich in fiber and show potential to bioenergy or macromolecule extraction. The composition of these materials is based on cellulose, hemicellulose and lignin. In brief, these macromolecules are interesting for several added value products, for instance, cellulose: used in fermentation to solvent and biofuels and cellulose-derived products such as carboxymethyl cellulose and cellulose nitrate; hemicellulose: used in fermentation to solvent and biofuels, biofilm, xylooligosaccharides; lignin: phenols, activated carbon, carbon fiber. Among several possible applications of sugarcane waste, the use in electricity generation is predominant, not considering its biotechnological potential.

Among biomass components, the hemicellulose is a special class of polysaccharides characterized by their diversity and heterogeneity. The hemicellulose is wide dispersed among plants, with different polysaccharides on hardwood (glucuronoxylan and glucomannan) and softwood (galactoglucomannan and arabino-glucuronoxylan) (Fengel and Wengels, 1984). For SCB, the hemicellulose has been reported as mainly composed by xylan polysaccharides. Furthermore, the most common hemicellulose polysaccharides are xylans, constituted of xylose units. The structure of the xylan content is highly dependent on the plant source and also can vary according to the sugarcane plant fractions (Brienzo et al., 2014). The materials rich in xylan are highly interesting to XOS production. The XOS can be produced from LCM using chemical methods, such as autohydrolysis, steam explosion, acid catalyzed medium, and enzymatic hydrolysis or a combination of these kinds of methods (Brienzo et al., 2010; Yang et al., 2005; Vázquez et al., 2000).

Xylooligosaccharides are not digestible in the human stomach and they are considered as prebiotic as they stimulate beneficial microorganisms in the human guts. The XOS are non-cariogenic and can be used as dietary

sweeteners as low-calorie diet food. The XOS are ingredients of functional foods, providing health benefits (Vázquez et al., 2000). Therefore, these sugars can improve the modulation of the colonic microbiota, especially bifidobacteria and lactobacilli (Vázquez et al., 2000; Nabarlantz et al., 2007; Gullón et al., 2008). Hence, the XOS are beneficial to the organism improving the bowel's function, calcium absorption, protection against cardiovascular diseases and also decreasing the risk of colon cancer, due to the formation of smaller fatty acids chains (Grootaert et al. 2007; Wang et al. 2009). Effects related to skin and blood, immunological action, anti-oxidant activities, anti-inflammatory and antiallergenic effects are also noted (Aachary and Prapulla, 2009). Despite all these health benefits, the XOS are poorly produced and consumed by humans and animals, because their cost is higher than other products in this same category. Therefore, more research is necessary to make the technology of XOS production more available and economical, especially when relating to prebiotic XOS.

Recently, the structure and health benefits of XOS have been reviewed (Carvalho et al., 2013). This review emphasizes the physiological properties and benefits for the body, such as health improvement through nutrient adsorption increase and factors related to inhibiting pathogenic microorganisms and consequences of a good gut development. XOS properties are dependent on its structure, which relies on the kind of sugars present and also on the number of sugars units (degree of polymerization - DP). Xylan is a polysaccharide that can be highly branched (acetyl groups, arabinose, galactose and feruloyl groups).

The market and consumption of oligosaccharides (including XOS) are currently gradually increasing. Nowadays, Japan is one of the biggest producers and consumers of oligosaccharides, with relevance to Asian countries. Furthermore, it is expected that, with the increase of health consciousness by the humans, the market of products with oligosaccharides will also increase. The human consciousness can be interpreted here as the importance and role of functional foods (added oligosaccharides) in the improvement of human health. Moreover, replacing the animal antibiotic uses for functional food should be considered as a serious issue, considering the negative effects of these compounds. The effect of antibiotic residues in meat for human consumption is not actually clear, but it is known that the overuse of antibiotics causes pathogens strains to increase (Carvalho et al., 2013). Considering the oligosaccharides as functional food ingredients of big importance and market prediction, there is a great interest in XOS production. Currently, researchers are dedicated to make the processes of production of

XOS cheaper, including studies to understand the physiological function and properties of XOS.

Focusing on the SCB as a resource of hemicellulose (xylan) and as feedstock for XOS production, the following discussion reviewed the chemical composition and properties of the SCB and its xylan. The key technology involved in the extraction of xylan and XOS production was discussed. The effectiveness and properties of these oligosaccharides were presented as well as their use as a prebiotic for humans and animals.

SUGARCANE BAGASSE AS XYLAN SOURCES

Sugarcane is a C₄ (a reference to a pathway in which CO₂ is first fixed into a compound that contains four carbon atoms) perennial sucrose-storing grass, belonging to the genus *Saccharum* which has its origins in Asia. Sugarcane is cultivated in tropical and subtropical countries throughout the world, including the bigger producers Brazil (721 million ton), India (347 million ton), China (123 million ton), and Thailand (96 million ton). The SCB is generated after the crashing process conducted for sucrose extraction. The sugarcane culm is based on internode (sucrose storage) intercalated by short nodes (transversal septa). These tissues are different on what refers to physicochemical properties, vascular bundle distribution and response to pretreatment (Brienzo et al., 2014). The SCB contains two types of fiber: the cellulose fiber of rind, and the pith of the stem. The pith fiber is composed mainly of parenchyma cells and the rind of sclerenchyma cells (Miller et al., 2012). These structures distributed in the SCB make the material heterogeneous and have different recalcitrance to biotechnological routes conversion.

The SCB is a fibrous material that corresponds to 15% of the sugarcane (dry basis). The chemical composition of SCB is based on the macromolecule cellulose, hemicellulose and lignin, with a low amount of extractives and ash (Table 1). Cellulose forms microfibrils compounding a network structure with hemicelluloses, encrusted by lignin. This structure is the base of the plant cell wall, which is highly organized and make the isolation of the macromolecules more difficult. The chemical composition of the cell wall can vary according to the type of biomass and plant fraction, with differences in the polysaccharides organization (Carpita and Gibeaut, 1993).

Cellulose is recognized as one of the most abundant organic compounds in the world. Approximately 35-50% of the dry mass of SCB is cellulose.

Cellulose is a linear homopolysaccharide, composed by β -D-anhydroglucopyranose units, which are linked by $\beta(1,4)$ -glycosidic linkages. Cellulose (and hemicellulose) is a structural polysaccharide of the plant cell wall, what justifies its resistance and defiance on separating it from hemicellulose and lignin. Cellulose chains are organized with intra- and intermolecular hydrogen bonds, forming microfibrils structures, what characterizes the highly ordered (crystalline) or less ordered (amorphous) regions (Fengel and Wengels, 1984). The cellulose content is higher in the external fraction of the sugarcane culm, comprising 50%, the internode contains 44% and the node 42% of cellulose (Brienzo et al., 2014). The cellulose content in SCB varies according to species (Table 1).

Hemicelluloses are the second most important polysaccharides in biomass. Hemicelluloses consist in short chains of branched heteropolysaccharides, composed of hexoses and pentoses. The main constituents of the pentoses are D-xylose and L-arabinose. The main constituents of the hexoses are D-glucose, D-galactose and D-mannose. The sugars D-manose, L-rhamnose and D-galactose are more scarcely reported and appear in a small amount in SCB xylan (Peng et al., 2009). The major hemicelluloses component of SCB is xylose-based. Hemicellulose comprises 22-36% of the SCB, defined as L-arabino-(4-O-methyl-D-glucurono)-D-xylan (Sun et al., 2004). The type and amount of hemicelluloses vary widely, depending on plant and its tissue type. SCB fractions have a different amount of hemicelluloses: 16, 27 and 29% of bagasse mass on the external fraction, node and internode, respectively (Brienzo et al., 2014). Xylan is the representative hemicelluloses of SCB since its composition is more than 80% of xylose in the backbone (Rocha et al., 2015; Brienzo et al., 2009). The xylose content in SCB has been reported as a range of 8.8 to 20.4%, to both classical and precision breeding SCB varieties (Benjamin et al., 2013).

Lignin is the second most abundant organic (aromatic) compound, after cellulose, found in the plant. Lignin is responsible for joining the cellulose fibers with the hemicelluloses network, providing rigidity to the cell wall. The lignin physical distribution surrounding the fibers protects the polysaccharides from chemical or physical extraction and microbial attack. This characteristic enhances the cell wall recalcitrance, making it difficult to isolate hemicellulose. Lignin is an amorphous macromolecule, defined as a three-dimensional polymer with a phenylpropane structure. The lignin structure is formed by enzyme-mediated radical coupling of the three monolignols that, in the molecule, are named p-hydroxy-phenylpropane (H), syringil (S) and guaiacyl (G) units. The ratio between lignin units is suggested to be related to

the material recalcitrance; the S unit is desirable to generate material with a lower resistance to the delignification process (Chiang and Funaoka, 1990). Lignin has been accepted to be responsible for the biomass recalcitrance and studies have shown that a good strategy is to select SCB feedstock with low lignin content for a better response to pretreatment and enzymatic digestibility (Brienzo et al., 2015). Lignin content varies according to the species in a range of 14-30% (Table 1). SCB fractions have a different amount of lignin: 29% in the external fraction and 22% in the internode and node (Brienzo et al., 2014). This difference of lignin content among these fractions could imply a different amount of hemicellulose extraction from each fraction.

The cell wall has other components that are not chemically linked to the cellulose-hemicellulose-lignin matrix, the extractive compounds. These substances are so named because they can be solubilized by solvents (ethanol, hexane, water, etc.). Extractive compounds comprise a large variation in the type and amount found in different species of SCB (Table 1). SCB extractives have been reported in a range of 3-14%, what depends on the species, growth condition and age. Among the compounds, phenolics are responsible for protection, avoiding insect, fungi and bacteria attack. The main types of compounds are phenolics, fats, fatty acids, resin, waxes and lignans. Since extractives are not linked to the lignocellulosic matrix they are removed previously of the biomass analysis such as chemical composition. Considering extractives removal, not for analytical purposes, their content is determined by extraction with ethanol and water in separate cycles, or with a mixture of cyclohexane/ethanol (1:1, v/v). The inorganic fraction of the biomass is named ash content. The ash is determined by burning biomass in mufla and quantifying it gravimetrically. The ash content in SCB varies on a short range around 2% (Canilha et al., 2011; Brienzo et al., 2009; Santos et al., 2011), however, a higher amount of ash has been reported (Table 1). SCB when in contact with soil in the field, normally shows a higher amount of ash, which comes from an excess of soil and dust (Szczerbowski et al., 2015). Samples withdrawn from the field should be washed with water at room temperature with the intent of removing these components that are not from the biomass composition. The biomass inorganic components are calcium, magnesium, silica, sodium, potassium, strontium, manganese and phosphorous (Szczerbowski et al., 2015). Considering the increasing of the uses of biomass to electricity generation through combustion, it is predicted that the amount of residual ash generated in such process will also increase. This ash will cause environmental concerns related to its properly storage and disposal. The

challenge in to using this waste will be to applying it as feedstock to high-value products.

Table 1. Sugarcane bagasse chemical composition

	Chemical composition (% , dry mass)					Reference
	Cellulose	Hemicellulose	Lignin	Extractives	Ash	
	46.2	27.8	22.1	-	1.0	Santos et al., 2011
	45.0	31.8	20.3	-	2.9	Zhao et al., 2009
	35.0	35.8	16.1	-	-	Sasaki, 2003
	39.6	24.0	22.4	5.0	1.3	Benjamin et al., 2013
	35.1	30.3	19.6	9.9	1.6	Benjamin et al., 2013
	40.7	31.5	14.4	7.4	0.8	Benjamin et al., 2013
	42.4	25.2	19.6	-	1.6	Brienzo et al., 2009
	47.3	26.4	14.1	11.4	-	Brienzo et al., 2015
	43.1	25.1	20.8	4.9	-	Brienzo et al., 2015
	40.9	27.4	14.6	8.4	-	Brienzo et al., 2015
	40.5	29.0	30.6	-	2.3	Zend et al., 2014
	45.5	27.0	21.1	4.6	2.2	Rocha et al., 2011
	45.0	26.4	23.4	2.7	2.5	Rocha et al., 2012
	36.8	28.3	24.7	6.8	3.1	Rocha et al., 2015
	45.6	26.5	21.4	3.6	2.9	Rocha et al., 2015
	44.7	28.8	19.5	3.2	3.6	Rocha et al., 2015
	38.5	27.8	17.7	2.7	8.8	Guilherme et al., 2015
	45.0	25.8	19.1	9.1	1.0	Canilha et al., 2011
	36.1	26.9	26.2	5.3	2.1	Saad et al., 2008
	44.4	22.9	17.5	14.1	-	Maryana et al., 2014
	45.0	31.8	20.3	-	-	Zhao et al., 2009
Minimum	35.0	22.9	14.1	2.7	0.8	
Maximum	47.3	35.8	30.6	14.1	8.8	
Average	42.0	27.7	20.0	6.4	2.4	

Cellulose (glucan): represented as sum of anhydromonomers of glucose;

Hemicellulose: represented as sum of anhydromonomers xylose, arabinose and acetyl group; Lignin: represented as sum of soluble and insoluble lignin; (-) not determined.

HEMICELLULOSE STRUCTURAL AND CHEMICAL PROPERTIES

Sugarcane bagasse hemicellulose is predominantly constituted of xylan, which is composed of xylose units in the backbone. SCB hemicellulose is defined as L-arabino-(4-O-methyl-D-glucurono)-D-xylan (Sun et al., 2004). Xylose accounts more than 70% in the xylan, in average, with a reported range of 43 to 93%. This polysaccharide shows branch groups of arabinose, acetyl groups and uronic acids (Table 2). Xylan backbone is made up of xylose linked by β -1,4 linkages with branch groups at the positions C2 and C3 with arabinosyl, uronic acids and acetyl groups (Sun et al., 2004). Arabinose is the branch group that appears in a higher amount. It is linked to xylan backbone by α -1,2 or α -1,3 linkage; galactose is linked by β -1,5 linkage to the xylan backbone. Xylan branch groups can be associated to other groups, aromatic feruloyl (from ferulic and diferulic acids) and p-coumaroyl can be attached to arabinose residues at the O-5 position (Saulnier et al., 1995). Other branch groups appear linked to xylan of SCB, which is reported to contain branch groups, what suggests that the xylan of this biomass is a heteropolysaccharide. SCB is a heterogeneous material, the fractions of the culm contain different amounts of xylan (Brienzo et al., 2014). Furthermore, it could be suggested that the xylan characteristic and properties could be different in the SCB fractions.

The branch groups of xylan of SCB depend on the variety type (Benjamin et al., 2014) and also on the condition of the extraction method (Brienzo et al., 2009). The contents of arabinose, galactose, glucose, rhaminose and uronic acids are quite different on literature reports (Table 2). The more branched chain correlates to solubility, which is important considering molecule reactivity. The branches also collaborate to chain characteristics, such as charge, which depends on the substituent groups. Some works showed a high content of glucose as part of the xylan; however, it could be probably from glucan and pectic polysaccharides (Geng et al., 2006). The use of water or a dilute alkaline medium before a more concentrated extraction probably removes the more branched xylan (Table 2). The use of peroxide in alkaline medium provokes the acetyl content removal from the xylan chain (Brienzo et al., 2009). The alkaline medium breaks alpha-ether linkages of lignin (of phenolic units), releasing the associated molecule that can be a hemicelluloses chain.

The alkaline medium also cleaves ester linkages, that include acetyl groups and ferulic and diferulic acids that link xylan chains to lignin. SCB with high acetyl content is positive for XOS release through pretreatments, such as autohydrolysis and steam explosion. These pretreatments, with no addition of catalyst, perform well in biomass with high acetyl content breaking xylan in XOS and xylose.

Hemicelluloses are naturally associated to cellulose and covalently linked to lignin in LCM. Xylan lignin residual content varies between 0.7 to 13% for extraction with hot water, alkaline and peroxide medium (Table 2). SCB pretreated with Soda-Anthraquinone has been further investigated to identify the structure formed of lignin-carbohydrate-complex (LCC) content. The LCC isolated were based on xylan-lignin complex and glucan-lignin-xylan (Njamela et al., 2013). The authors also showed that the ester linkages were broken during the hemicelluloses extraction. The isolation of xylan with alkali or peroxide in alkaline medium can show impurities related to lignin association. The association of lignin and hemicelluloses is done by several types of linkages that are resistant to alkaline or peroxide action. SCB has hydroxycinnamic acid (ferulic, coumaric and sinapic acids) involved in cross-linking xylan and lignin molecules (Rose, 2003). SCB xylan can be esterified to lignin. The hydroxyl groups of lignin units can be linked to carboxylic acid groups of uronic acids (Rose, 2003).

Native or extracted xylan contains molecules with a different number of xylose units, what means different degrees of polymerization (DP). The DP is defined as the number of xylose units in a xylan molecule. Xylan is reported as being made of anhydroxylose units linked by β -1,4 linkages with a DP up to 200. The xylan molecule is generally partially cleaved during the extraction process, with a consequent reduction in the DP. The DP values of xylan can vary according to the process method, its severity and the evaluation method (centrifugation, gel permeation, light scattering). The polysaccharide molecular mass (g/mol) is defined as the DP multiplied per the anhydroxylose molecular mass. The SCB xylan molecular mass has been reported in a wide range, including values between 6500 to 86000 g/mol (Table 2).

Table 2. Sugarcane bagasse hemicellulose chemical composition and extraction methods

Xylan composition (% dry mass)								Molecular mass (g/mol)	Extraction yield (%)	Extraction condition	Reference
Xyl	Ara	Gal	Man	Rha	Glu	Uronic acids	Lignin				
50.4	4.4	-	-	-	3.9	4.4	10.0	21100	52.0	H ₂ O ₂ in alkaline medium (2%, m/v), 20°C, 4h.	Brienzo et al., 2009
74.9	6.0	-	-	-	6.4	5.2	9.6	21100	60.8	H ₂ O ₂ in alkaline medium (6%, m/v), 60°C, 16h.	Brienzo et al., 2009
78.0	5.8	-	-	-	5.4	6.5	10.4	22185	94.0	H ₂ O ₂ in alkaline medium (4%, m/v), magnesium sulfate (0.25% m/m), 40°C, 10h.	Brienzo et al., 2009
93.2	5.6	0.2	0.4	0.2	0.5	2.3	6.1	40770	-	Successive water, 1 and 3% NaOH, 50°C during 3h, precipitation with 15% ethanol.	Peng et al., 2009
91.0	6.4	1.3	0.3	0.1	1.1	2.1	4.5	86720	-	Successive water, 1 and 3% NaOH, 50°C during 3h, precipitation with 30% ethanol.	Peng et al., 2009
87.0	7.8	1.1	nd	nd	3.3	1.7	2.2	77140	-	Successive water, 1 and 3% NaOH, 50°C during 3h, precipitation with 60% ethanol.	Peng et al., 2009
82.0	9.3	0.7	1.0	3.0	4.1	4.8	3.1	35200	55.5	1 M NaOH, 20°C, 18h	Xu et al., 2006
81.0	9.8	0.5	1.1	4.9	2.6	5.8	2.7	35820	59.1	1 M NaOH, 30°C, 18h	Xu et al., 2006
78.0	11.6	0.3	1.4	6.5	2.2	5.6	2.4	37430	62.1	1 M NaOH, 40°C, 18h	Xu et al., 2006
43.1	25.1	2.7	4.0	0.7	9.8	2.1	13.3	6500	3.0	Hot water, 170°C, 15 min.	Banerje et al., 2014
69.0	12.1	1.4	1.1	0.1	9.7	1.7	6.3	22500	8.1	Hot water, 200°C, 15 min.	Banerje et al., 2014

Table 2. (Continued)

Xylan composition (% dry mass)								Molecular mass (g/mol)	Extraction yield (%)	Extraction condition	Reference
Xyl	Ara	Gal	Man	Rha	Glu	Uronic acids	Lignin				
78.8	4.6	1.0	0.6	0.1	3.1	3.2	7.2	27900	14.9	Hot water extracted material 15 min, 170°C, and 3% H ₂ O ₂ , 0.25% magnesium sulfate, 40°C, 12h.	Banerje et al., 2014
83.4	5.7	0.9	0.4	0.2	3.0	3.4	3.9	12100	12.2	Hot water extracted material 15 min, 200°C, and 3% H ₂ O ₂ , 0.25% magnesium sulfate, 40°C, 12h.	Banerje et al., 2014
55.2	10.7	7.6	4.1	1.8	20.4	7.0	9.6	7380	4.1	Water extraction 55°C, 2h.	Sun et al., 2004
68.6	12.7	1.9	-	-	7.3	3.5	0.7	23340	9.6	Successive extractions of the 0.5 M NaOH treated bagasse with 0.5% H ₂ O ₂ , 55°C, 2h.	Sun et al., 2004
76.5	14.7	3.0	0.3	1.0	13.0	4.8	4.2	38890	9.6	Successive extractions of the 0.5 M NaOH treated bagasse with H ₂ O ₂ (0.5; 1; 1.5; 2; 2.5 and 3%), 55°C, 2h.	Sun et al., 2004
81.3	11.9	1.7	0.5	0.5	4.0	3.5	5.1	45370	12.0	0.5 M NaOH at 55°C, 2 h from the water treated bagasse.	Sun et al., 2004
43.1	4.4	0.2	0.3	0.1	0.5	1.7	0.7	6500	3.0	Minimum	
93.2	25.1	7.6	4.1	6.5	20.4	7.0	13.3	86720	94.0	Maximum	
74.8	9.7	1.7	1.3	1.6	5.9	4.0	6.0	33026	32.6	Average	

(-) not detected or determined; Xyl: xylose; Ara: arabinose; Gal: galactose; Man: mannose; Rha: rhamnose; Glu: glucose.

XYLOOLIGOSACCHARIDES PREBIOTIC POTENTIAL AND HEALTH BENEFITS

Xylooligosaccharides are oligomers comprised of xylose units containing β -1,4 bonds. They are usually marketed as a white powder containing 2 to 12 xylose units (Figure 1), although up to 20 units molecules are still considered XOS (Mäkeläinen et al., 2010a; Moure et al., 2006; Vázquez et al., 2000; Kabel et al., 2002). The composition and stability of XOS are related to the type of oligosaccharide, sugar residues, bonds, ring formation and anomeric configurations and the extraction process (Kabel et al., 2002; Carvalho et al., 2013). The XOS present some advantages compared to fructooligosaccharides and inulin, such as resistance to a broad range of pH (2.5-8.0) and stability at high temperatures, above 100°C. The lack of the enzyme that cleavages β bonds in humans and the stability at acidic pH allow the XOS to reach the intestines intact, serving as a substrate for beneficial bacteria colonizing the intestinal tract (Bielecka et al., 2002). This feature allows the XOS to be classified as non-digestible oligosaccharides (NDOS), which are soluble fibers. The NDOS are water soluble and generally present sweetness (Crittenden and Playne, 1996; Manning and Gibson, 2004).

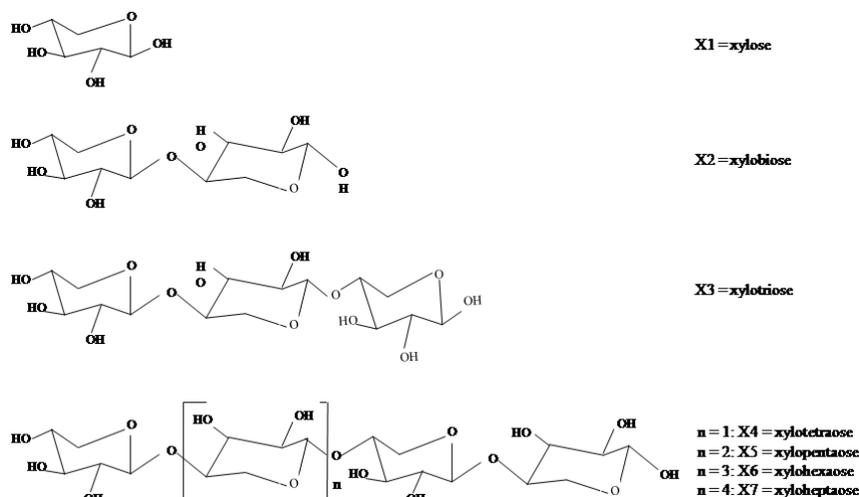


Figure 1. Schematic structure of xylose and xylooligosaccharides.

The gastrointestinal tract is heavily colonized by various microorganisms, including bacteria with pathogenic potential, as well as beneficial bacteria. Due to their beneficial effects, and especially due to the ability to selectively stimulate the growth of *Bifidobacterium* and *Lactobacillus*, XOS are considered prebiotic compounds (Moure et al., 2006, Mussatto e Mancilha, 2007). Many prebiotics are commercialized worldwide, however this requires that the prebiotic be resistant to gastric juice and to digestive enzymes present in the gastrointestinal tract; able to be fermented by the intestinal flora; and selectively stimulate the growth or activity of intestinal bacteria beneficial to health (Gibson et al., 2004).

Nowadays, a great interest in a healthier lifestyle has risen, once the development of foods that bring health benefits has become the main objective of the food industries. XOS can be used for the production of xylitol (sweeteners), that act in the prevention of tooth decay and can be added to food for consumption by people with diabetes or in need of low calorie diets (Rivas et al., 2002). Apart from the prebiotic effect, studies indicate improvements in bowel function, an increase in the absorption of nutrients, fortification of the immune system, prevention of cardiovascular diseases, reduction of colon cancer risk and the triggering of allergenic, anti-inflammatory and anticariogenic activity (Grootaert et al., 2007; Wang et al., 2009; Aachary and Prapulla, 2009).

Another aspect of XOS usage is related to animal feeding. The use of antibiotics in livestock aims to fight infections and help animals gain weight. However, the excessive use of antibiotics enables resistance and production of virulent strains (Carvalho et al., 2013). In general, the use of prebiotics as an additive in animal feeding has attracted attention due to their effects on animal health. The addition of prebiotics on chicken ration is gaining prominence as organic additives since it shows positive effects similar to antibiotics, but without causing bacterial resistance (Flemming and Freitas, 2005). Studies *in vitro* demonstrated the positive effects of XOS digestibility in the intestine of ruminants, since they stimulated the growth of probiotic strains (Samanta et al., 2012). Beneficial changes in the intestinal microbiota reduce the population of some potentially pathogenic strains of the genus *Escherichia*, *Salmonella*, *Enterobacteriaceae* and *Streptococcus*, bacterial agents of intestinal disorders and toxins release (Holzapfel, 1998). Prebiotics are also studied because of health benefits they provide during pregnancy. Studies *in vivo* with rats fed with a combination of FOS and XOS showed that the supplementation with prebiotics during gestation can protect the fetus,

developing brain against oxidative stress and also restoring enzymic antioxidants and mitochondrial function (Krishna et al., 2015).

The studies of XOS biological properties and prebiotic effects have been done *in vitro* and *in vivo* for humans and animals. The assay *in vitro* considers microorganisms common in the human and animal guts, evaluating the potential of stimulating the beneficial and inhibiting the pathogenic microorganisms. Recent tests *in vitro* showed that XOS derived from corn cobs improve the growth of *Lactobacillus plantarum* S2, and the combination of both prebiotic and probiotic causes inhibiting effects, decreasing the viability of *Enterococcus*, *Enterobacter*, and *Clostridia* spp. This combination also showed an antioxidant activity better than that of XOS or *L. plantarum* alone (Yu et al., 2015). Several species of *Bifidobacterium*, *Lactobacillus brevis* and some *Bacteroides* are able to grow in culture media containing XOS with 2-5 units of xylose as a sole carbon source. Moreover, XOS can inhibit *Escherichia coli*, *Enterococcus* spp., *Clostridium perfringens* and *Clostridium difficile* (Crittenden et al., 2002). Studies on pure cultures and on simulated colon models showed that XOS selectively stimulate the growth of *Bifidobacterium lactis* and some species of *Lactobacillus* (Mäkeläinen et al., 2010a, b). XOS are also able to inhibit the adhesion of *Listeria monocystogenes* on the intestinal epithelium (Erbesbach et al., 2011). Therefore, the quality of the produced XOS is important when considering the prebiotic effect.

Commercial XOS (Wako Chemicals) and the one produced from *Miscanthus giganteus*, fermented *in vitro* with human fecal microbiota, demonstrated an increase in bacteria of genus *Bifidobacterium*, *Lactobacillus* and *Escherichia* (Chen et al., 2016a). Another work using XOS produced from *Miscanthus giganteus* demonstrated that *Bifidobacterium adolescentis* and *Bifidobacterium catenulatum* grew in XOS, and acetic and lactic acids were the products of this fermentation (Chen et al., 2016a). Commercial XOS (Xylooligo 95P; Suntory, Osaka, Japan) produced by autohydrolysis, containing 83% of xylobiose and xylotriose were used in culture medium as a carbon source. These XOS were responsible for higher growth of *Bifidobacterium* strains (*B. adolescentis* and *B. longum*) than the culture with medium formulated with another XOS with higher DP and containing only 24–41% of xylobiose and xylotriose. Furthermore, XOS with DP 5 and 6 (longer chain lengths) reduced the degree of consumption of these oligosaccharides by the bacteria (Moura et al. 2007).

Xylooligosaccharides dosage and DP are important issues that have been investigated by several researchers. The key factor for the use of XOS in

human and animal feed is to determine the required daily dosage and the best range of DP to maximize its effects. Tests carried out *in vitro* and *in vivo* using oral administration of XOS (DP 2 and 3, with xylose) of 5g/day for 3 weeks indicated that XOS reached the intestine intact and were able to stimulate the growth of bacteria of the *Bifidobacterium* genus, particularly *B. adolescentis*. Furthermore, XOS inhibited the growth of *Clostridium* and *E. coli* (Okazaki, 1990). Studies *in vivo* showed that a low dose of commercial XOS (Shandong Longlive Bio-Technology Co., Ltd., China.) is enough to increase *Bifidobacterium* in the human guts. Only 1.4 g/day increased the counts of *Bifidobacterium* compared to a placebo group, and 2.8 g/day showed an even greater increase, without any gastrointestinal side effects (Finegold et al., 2014). This study did not observe changes in the *Lactobacillus*, *Clostridium* and *Enterobacteriaceae* counts, or in pH and short chain fatty acids related to XOS consumption. The maximum tolerant dose of XOS consumption for humans was determined as 12 g a day. With this amount of daily XOS, side effects such as diarrhea and constipation were observed to be similar to those subjects who didn't take any XOS (Xiao et al., 2012).

HEMICELLULOSE EXTRACTION METHODS

The hemicellulose extraction method depends on the purpose and application. The selection of the extraction method should be based on the desirable final characteristics of the hemicellulose. Among these important characteristics for application are the degree of polymerization, degree of branching/substitution, impurity such as residual lignin content, solubility and reactivity. Considering hemicellulose recovery based on polysaccharide or monosaccharide form, alkaline or acid concepts can be successfully applied. Hemicellulose extraction with acid medium and process derived from it lead to a final product based on monosaccharide and oligosaccharides. On the other hand, hemicelluloses extraction with alkaline medium and process derived from it lead to a final product based on polysaccharides with a high degree of polymerization. Considering XOS production, both methods could be applied. Acid medium results in low XOS yield high xylose yield, with by-products formation. Alkaline extracted hemicelluloses require a second step for XOS production, which could be an acid or an enzymatic hydrolysis. Despite the many possibilities for XOS production, it is still not economic and technically feasible for industrial scale (Carvalho et al., 2013).

Pretreatment is necessary in order to remove/modify lignin in LCM and to obtain sugars from cellulose or hemicellulose. This process is then followed by enzymatic hydrolysis to break the polysaccharides and consequently release the oligomers or monomers (Mosier et al., 2005; Balat et al., 2008). The pretreatment may act by altering structural factors or chemical composition of the biomass, besides increasing the surface area, facilitating the accessibility of the constituent LCM (Brienzo et al., 2015; Mosier et al., 2005). The chemical inhibitors may be present in the biomass or produced during the pretreatment, generally in severe conditions pretreatments. The sugars degradation forms organic acids that can be regarded as enzymatic hydrolysis inhibitors (Carvalho et al., 2015). The toxic compound 5-hydroxymethylfurfural (HMF) is derived from the degradation of hexoses, as furfural comes from pentoses. The breaking of furfural and HMF releases formic acid, in addition, HMF degradation produces levulinic acid, and phenolic compounds are formed from the lignin (Rasmussen et al., 2014). After the pretreatment step, alcohols and ketones are used for the recovery of solubilized hemicellulose (Akpınar et al., 2009; Vázquez et al., 2005). However, XOS obtained through certain methods need a more complex purification step due to the by-products and xylose present in the hydrolysate.

ACID AND HYDROTHERMAL PRETREATMENT

The acid hydrolysis is often performed with diluted acid to prevent corrosion of equipments and high energy demand for the acid recovery. The pretreatment with diluted acid results in less formation of furfural and hydroxymethylfurfural (HMF) (Gómez et al., 1985). The most commonly used acids in this process are sulfuric, hydrochloric, acetic and phosphoric acids. In this pretreatment, hemicellulose is solubilized, containing predominantly pentose and a fraction of an insoluble part, that contains mainly cellulose and lignin. The acid concentration should be low, in order to avoid hemicellulose hydrolysis into monomers, such as xylose, and consequently the release of compounds from its degradation, like furfural.

Acid hydrolysis is able to cleave the glucosidic linkage, fractionating the polysaccharides to oligosaccharides or monosaccharide (Figure 1). The acid catalysis occurs in three steps: a rapid protonation of the oxygen atom of the ether linkage (C-O-C); the transference of the positive charge to the C1, forming a carbocation ion and simultaneous split of the ether linkage; and a fast addition of water to the carbocation ion. The β -1,4 linkage in xylan is

susceptible to acid attack, provoking chain breakdown, which depends on the acid type and concentration, and reaction condition, such as temperature and time. The acid-catalyzed xylan chain hydrolysis can happen through random attack and depolymerization. The random cleavage breaks the chain, decreasing the degree of polymerization and releasing oligosaccharides. The depolymerization depends on the chain DP and releases monosaccharides. These two types of acid attack occur together and need to be controlled to maximize the desired product: xylose or XOS.

Hydrothermal pretreatment is catalyzed by steam or water hydronium (H_3O^+), process known as hydrothermolysis, autohydrolysis or hotwater (Figure 2). Autohydrolysis can be carried out at temperatures between 150 and 220°C. This process has the advantage of not using harsh chemicals to extract hemicellulose, avoiding corrosion of the equipment. This process is mainly applied in agro-industrial waste, in which hemicelluloses are mainly xylan (Overend and Chornet, 1989). The amount and concentration of solubilized products distinguish autohydrolysis in hydrothermal pretreatment and steam explosion. Steam explosion is performed by the sudden decompression of a pressurized system containing high pressure water steam and biomass (Hendriks and Zeeman, 2009). The amount of products from the degradation of sugars is smaller in the hydrothermal pretreatment than in steam explosion (Tahezadeh and Karimi, 2008).

The autohydrolysis is a process with no catalyst-added, and the process occurs under acidic condition, due to acetic acid released by cleavage of the acetyl groups substituent in the xylan chain. The acetic acid released creates a slight acid medium with pH around 4, and its release is a temperature-based reaction of ester linkages, which are unstable at high temperature (Fengel and Wengels, 1984). The severity of the autohydrolysis process is determined by the temperature and the reaction time. These two variables are directly related to the acetyl groups originated from hemicellulose (Garrote, 1999). Considering that the acetyl groups on hemicellulose are responsible for acidifying the medium, it could be suggested that the amount of acetyl on the biomass will lead to different responses on XOS production (Nabarlatz et al., 2007). Considering the autohydrolysis process for XOS production, the feedstock material characteristics are important for the final yield. The autohydrolysis is successful for biomass with high acetyl content. On the other hand, acid catalyzed could be desired for biomass with low acetyl content. The water dissociation to form hydronium has been suggested, however the main factor involved in this ion formation is the acetic and uronic acids released from the biomass (Nabarlatz et al., 2004).

The hydrothermal pretreatment, catalyzed or not, releases xylan from the biomass in the form of soluble XOS, which are in sequence breakdown on xylose. Depending on the reaction condition (severity, i.e., temperature, reaction time and pH), the xylose can be degraded to furfural, which can be converted into other degradation and condensation products (Rasmussen et al., 2014). The xylan solubilization and hydrolysis to XOS, xylose and subsequent conversion to degradation products (by-products) could occur in a sequence of consecutive reactions (Figure 3). However, the LCM is heterogeneous and the interaction between xylan and the other macromolecules can be different, considering different material fractions and cell wall organization. The SCB has fractions with different xylan contents (Brienzo et al., 2014), and it is possible that its recalcitrance is different, implying fast and slow reaction for XOS and xylose releasing.

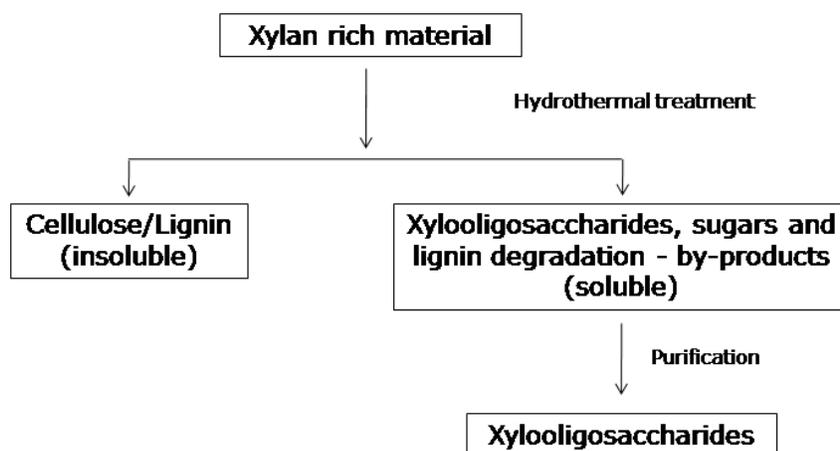


Figure 2. General scheme for XOS production by hydrothermal treatment.

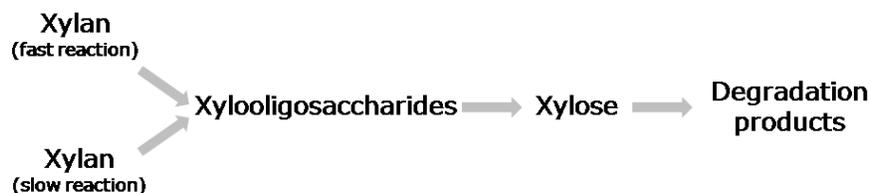


Figure 3. Xylan acid hydrolysis and possible steps. A complex scheme should be developed considering the recalcitrance and material heterogeneity. The xylan can be at different degrees: soluble, acetyl content, branches, accessible and lignin protected.

Hydrothermal pretreatment has been described as efficient for XOS production because of high yield (Otieno and Ahring, 2012; Chen et al., 2014, Moniz et al., 2014). On the other hand, there is a production of undesirable compounds, such as monosaccharides (xylose) and the degradation products of sugars and lignin. The releasing of by-products and monosaccharides requires a purification step, which accounts on process cost, considering industrial scale. Carbon adsorption could recover 45% of XOS, while serial ion exchange resin treatments could recover 91% of XOS (Chen et al., 2016b). A hydrothermal pretreatment acid catalyzed (0.1% of H₂SO₄, pre-soaked at 60°C/12 h, followed 145°C/1 h) was efficient for several biomass, releasing 92% of XOS from SCB (Otieno and Ahring, 2012). These XOS showed DP ranging from 2 to 25, with higher presence of DP 2 to 5. Hydrothermal pretreatments are dependent on the severity factor, which should be adjusted to maximize XOS release. A process with low severity is characterized by low XOS, however, with low by-products release. High severity also is characterized by low XOS production, because of its breakdown, but high xylose and by-products release (Moniz et al., 2014).

ALKALINE HEMICELULOSES EXTRACTION

The alkaline extraction breaks down the cell wall, disrupting the macromolecule components, so making xylan and lignin be solubilized. The reaction normally uses a strong solution of NaOH, KOH, Ca(OH)₂, NH₃ and also oxidative agents, i.e., H₂O₂ in alkaline medium. The effectiveness of the xylan solubilization depends on the lignin content in biomass. The alkaline action is assigned to a saponification of the ester linkage, separating the hemicellulose from the lignin, and releases uronic and acetic acid. In addition, the pretreatment of the biomass with a solution of NaOH causes a swelling of the cellulose structure and accordingly increases the surface area (Weil et al., 1994). This increased structure facilitates the intake of water, which acts disrupting the interaction between lignin and carbohydrates. The result is a decrease in crystallinity, and the rupture of the lignin that is soluble in these conditions (Weil et al., 1994; Balat et al., 2008). Compared to acid pretreatment methods, the alkaline methods have the advantage of removing the lignin without degrading the other components, usually due to milder process conditions, which can be carried out even at room temperature (Balat et al., 2008).

The H_2O_2 reacts with the aromatic and aliphatic structures of lignin, and it is widely used in pulp bleaching processes. The action of this oxidant agent can generate degradation products due to the formation of oxygen radicals. The influence of pH has been extensively studied and the literature shows the value of 11.6 (Yang et al., 2002). In alkaline medium, the hydrogen peroxide decomposes in hydroperoxide anion (HOO^-), the main agent in the degradation of lignin compounds. On the other hand, when the peroxide is used as the delignification agent in alkaline extraction, the decomposition occurs due to the formation of hydroxyl and oxygen, which begins lignin breakdown. Although the decomposition of peroxide is necessary for delignification to occur, the rate of decomposition must be reduced to avoid excessive formation of free radicals, which can lead to degradation of polysaccharides. The peroxide pretreatment can be favored by the removal of heavy metals with chelating agents, and adding later magnesium ions. The metals catalyze the decomposition of peroxide on hydroxyl radicals, provoking polysaccharide depolymerization and decrease the final yield; the magnesium ions stabilize the peroxide avoiding degradation (Thakore et al., 2005; Brienzo et al., 2009).

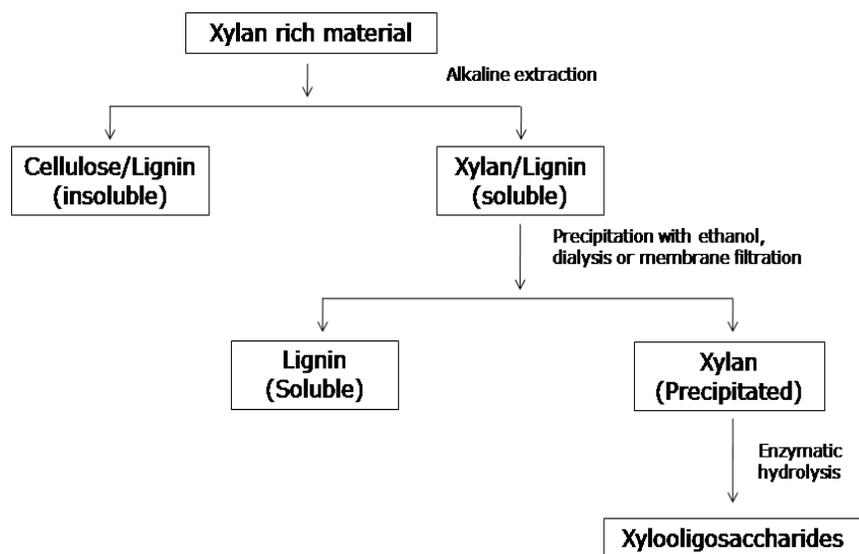


Figure 4. General scheme for XOS production by two-stage: hemicelluloses extraction and enzymatic hydrolysis.

Depending on the process condition, a high yield of hemicellulose can be extracted (Table 2). Once hemicellulose is isolated, the enzymatic hydrolysis act efficiently release XOS. This process is characterized in two-stage: an alkaline extraction of the hemicelluloses followed by an enzymatic hydrolysis (Figure 4).

ENZYMATIC HYDROLYSIS

Referring to lignocellulosic materials (LCM), enzymatic hydrolysis is a widely studied process, since this type of hydrolysis presents the specificity of the reaction and absence of secondary reactions, which would have decreased the yields. Another important characteristic of this hydrolysis is the lack of by-products formation, since the reactions happen mildly and there is no need of high pressures or temperatures, nor of the use of corrosive materials (Figure 4). However, the conversion of LCM into sugars through an economic process is still a challenge. The demand for hydrolytic enzymes is growing faster than ever and has become a driving force for researches on xylanases and cellulases. However, the production costs and low yields are challenges for industrial applications. The incorporation of economical sources (LCM such as SCB, wheat straw, corn cobs) in the culture medium for the growth of microorganisms and enzyme production may help decrease the production cost of the enzymes and special microorganisms can also be used as a biological pretreatment of LCM. Consequently, several processes are being developed using agro-industrial residues for the production of these enzymes (Lakshmi et al. 2009). Moreover, the bioconversion of these substrates may help the reduction of the environmental impact caused by the accumulation of waste (Camassola and Dillon 2009).

Although decades have already been dedicated to LCM conversion, the substrate characteristics that give more hydrolysis efficiency are still not completely understood. The enzymatic hydrolysis requires direct contact between the enzyme and the substrate. The enzyme must overcome physical barriers to adsorb on the surface of the substrate and finally catalyze the hydrolysis. LCM is naturally resistant to enzyme or microorganism action due to its highly organized cellulose-hemicellulose-lignin structure in the plant cell wall (Sant'Anna et al., 2014). Substrates with high lignin content and highly crystalline have low enzyme effectiveness. The factors that influence the performance of enzymatic hydrolysis process depend on the: (1) substrate structure, which is influenced by the substrate/material characteristics; (2)

interactions of the enzymes with the substrate, that depend on the nature and source of the enzyme complex. In addition to these factors, the enzymatic hydrolysis is influenced by the heterogeneity of the LCM. A better enzyme action could be reached with a previous isolation of the polysaccharide. Enzyme applied to XOS production requires hemicellulose extraction for further hydrolysis, characterizing a two-stage process (Brienza et al., 2010).

The hydrolysis of hemicellulose takes place through the action of endo-enzymes that act internally in the main chain and exo-enzymes, responsible for the cleavage that produces monosaccharides and oligosaccharides (Kalogeris et al., 2001). The endo- β -1, 4-D-xylanase (EC 3.2.1.8) acts on the main chain of xylan and generates XOS with low DP, which are substrates for exo- β -1,4-xylanase (EC 3.2. 1:37), that act on the non-reducing terminal D-xylose (Beg et al., 2001, Lakshmi et al. 2009). Hemicellulose normally is a heteropolymer with several branching units linked to the backbone. For the complete hydrolysis of the hemicelluloses different enzymes are necessary in synergistic action. Enzymes that act on the branches (dibranching or accessory enzymes) are arabinofuranosidase (EC 3.2.1.55), able to remove arabinose. α -glucuronidase (EC 3.2.1.131) removes glucuronic and acetyl xylan (Lagaert et al., 2014). Acid esterase (EC 3.1.1.72) removes acetyl groups (Juhász et al., 2005). According to enzymes action, for XOS production, it is desired an enzymatic pool with no exo- β -1,4-xylanase activity. The presence of this last enzyme will provide a release of xylose, decreasing the yield of XOS.

The presence of dibranching enzymes can lead to generate XOS with no branch units. Branched XOS can be released with enzymatic hydrolysis free of accessory enzymes (Puchart and Biely, 2008). In fact, the branch groups influence on the xylan hydrolysis rate, that is strongly dependent on the amount of branch units (Li et al., 2007). The endo-xylanases act differently on the xylan backbone, once they are influenced by the branch groups. Endo-xylanase from Glycoside Hydrolase (GH) Family 10 produces XOS with lower DP than GH family 11 (Maslen et al., 2007). The presence of branching can be a barrier, depending on the enzyme restriction due to the mode of action. Xylanase from *Aspergillus niger* hydrolyzes better the unbranched xylan, while xylanase from *Trichoderma longibrachiatum* can hydrolyze both types of xylan (Akpınar et al., 2009). Xylanases purified from *A. niger* could hydrolyze xylotriose, but is not able to hydrolyze arabinoxylotriose (Takenishi and Tsujisaka, 1975). The presence of substituents, which are typically located in the non-reducing terminal of XOS, protects the glycosidic bond of the main chain (Contat and Joseleau, 1981). The presence of branch groups can result in diverse biological properties. The branched XOS has shown a higher

selectivity by bifidobacteria compared to none branched (Van Laere et al., 1997). Currently, there is a lack of information and studies dedicated to elucidate the mechanism of the XOS and branched-XOS on the biological properties. XOS can be responsible for several biological properties different from the prebiotic effects: immunomodulatory, anti-cancerous, anti-microbial and growth regulator. Some of the properties are suggested to be related to XOS branch groups, for example, antioxidant activity is due to phenolic substituents as a branch; anti-allergy and anti-inflammatory properties are attributed to uronic substituents (Yuan et al., 2004; Izumi et al., 2004).

The optimization of the conditions of hemicellulose hydrolysis into XOS by xylanases is also another important step to improve the XOS technology, since a higher yield is necessary to obtain an economic bioprocess (Brienzo et al. 2009 and 2010; Akpınar et al. 2009; Carvalho et al. 2013; Carvalho et al. 2015). In addition, the presence of a low amount of β -xylosidase (β -1,4-xylan xylohydrolase, EC 3.2.1.37) in xylanase extracts is essential, since this enzyme converts xylobiose and other XOS into xylose (Vázquez et al. 2000). Some microbiological and biochemical aspects of xylanase hydrolysis can influence the XOS production. The improvement of the enzymatic reaction can be performed to obtain higher XOS yield and also reach a concentration by optimization of the dosage of xylanase, reaction time, temperature and substrate concentration. The hemicellulose studies with various extraction methods and followed hydrolysis revealed a maximum XOS concentration of 7.5 g/L using 2% of hemicellulose obtained from bagasse, 120-500 U/g xylanase during 48-72 h (Carvalho et al, 2015); 5.7 g/L of XOS after 96 h, 60 U/g using 2.6% of hemicellulose (Brienzo et al. 2010) and 1.7 g/L after 8 h (Jayapal et al. 2013). The XOS production with other substrates reached maximum production with corncob, 5.8 g/L after 24 h (Aachary and Prapulha, 2009) and 6.7 g/L after 8 h (Chapla et al. 2012).

The enzymatic hydrolysis process was suggested as the best option for XOS production, considering the release of a low amount of monosaccharides and undesirable products (by-products) (Aachary and Prapulla, 2009). The xylanases from *A. fumigatus* M51 reached a high level of XOS production (37.6%) after 48–72 h using hemicellulose extracted from SCB. This yield represents 68.8 kg of XOS with prebiotic activity (xylobiose and xylotriose) per metric ton of SCB (Carvalho et al. 2015). However, the best method for XOS production is not defined, since the enzymatic process is lower and probably less economic than the combination of acid and hydrothermal method. On the other hand, the enzymatic method resulted in the production of XOS with a degree of polymerization of 2–3, without by-products, while

hydrothermal pretreatment produced other XOS with higher DP without prebiotic effect, and some by-products, such as furfural and hydroxymethylfurfural (Otieno and Ahring, 2012; Chen et al., 2014, Muniz et al., 2014).

CONCLUSION

The worldwide current concern about the environmental protection leads to the biotechnological use of LCM. SCB is one of the most important wastes generated from the agro-industrial process. Among several possibilities of the SCB use, the hemicellulose extraction to be used as feedstock for high-value molecule production has gained attention. Hemicellulose has been studied for several applications in the field of energy, food and pharmaceutical products. Among them, the production of XOS has received special attention because of its prebiotic effect and for being considered as an ingredient in functional foods. They are favorable for the health of consumers as they are not cariogenic, have low calorific value and stimulate the growth of beneficial bacteria in the colon, as well as several biological properties can be provided. The hemicellulose extraction is not a simple task and still has challenges to reach the industrial application. The available process for hemicellulose extraction has to solve problems such as high amount of chemicals used, low extraction yield, sugars degradation and residual lignin. Further, the XOS generation through hydrothermal and enzymatic hydrolysis processes needs to be improved to overcome the material recalcitrance. The hydrothermal pretreatment has the challenge of avoiding sugars degradation products, and the control of hemicellulose depolymerization, avoiding xylose release or no prebiotic high DP XOS production. Despite the improvement in the technology of enzymatic hydrolysis, with the predominant production of prebiotic XOS and no toxic compounds generated, this process remains expensive, and it depends on the hemicellulose pre-extraction. The growing importance, biological properties and demand for XOS are opportunities for process development, leading to efficient hemicellulose extraction and enzymatic hydrolysis processes, or a safe hydrothermal process for XOS production.

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