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BIOTECHNOLOGY METHODS

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Thermopressurized diluted phosphoric acid pretreatment of ligno(hemi)cellulose to make free sugars and nutraceutical oligosaccharides

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Abstract Ligno(hemi)cellulosics (L(h)Cs) as sugarcane bagasse and loblolly pine sawdust are currently being used to produce biofuels such as bioethanol and biobutanol through fermentation of free sugars that are often obtained enzymatically. However, this bioconversion requires a pretreatment to solubilize the hemicellulose fractions, thus facilitating the action of the cellulolytic enzymes. Instead of the main free monosaccharides used in these current models, the modulation of thermopressurized orthophosphoric acid as a pretreatment, in the ranges of 3–12 atm and pH 1.5–2.5, can produce nondigestible oligosaccharides (NDOS) such as xylo-oligosaccharides (XOS) because heteroxylan is present in both types of hardwood and softwood hemicelluloses. A comparative thin-layer chromatographic analysis of the hydrolytic products showed the best conditions for NDOS production to be 7 atm/water, pH 2.25 and 2.50, and 8.5 atm/water for both sources. Particular hydrolysates from 7 atm (171 °C) at pHs 2.25 and 2.50 both for cane bagasse and pine sawdust, with respective oligosaccharide contents of 57 and 59 %, once mixed in a proportion of 1:1 for each plant source, were used in vitro as carbon sources for *Bifidobacterium* or *Lactobacillus*. Once both bacteria attained the stationary phase of growth, an unforeseen feature emerged: the preference of *B. animalis* for bagasse hydrolysates and, conversely, the preference of *L. casei* for pine hydrolysates. Considering the fact that nutraceutical oligosaccharides from both hemicelluloses correspond to higher value-added byproducts, the technology using a much diluted thermopressurized orthophosphoric acid pretreatment becomes an attractive choice for L(h)Cs.

Keywords Bifidobacteria · Lactobacilli · Galactoglucomannan oligosaccharides · Xylo-oligosaccharides

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Introduction

Ligno(hemi)cellulosics (L(h)C), such as agricultural, industrial, and forest residues, account for the majority of the total biomass present in the world, estimated at approximately 180 million tons per year [16]. These materials are generally composed of 40–50 % cellulose (water and alkali-insoluble fibers of β-1,4-glucan), 20–30 % hemicelluloses (noncellulosic polysaccharides, including alkali-soluble xylans, mannans, and glucans), 20–25 % lignin (a complex polyphenolic structure partially soluble in organic solvents), and 5–8 % extractives [6, 17]. The various types of L(h)C raw materials include sugarcane bagasse, wheat and rice straws, palm bunches, corncobs, soy hulls, and pine and other wood sawdust with varying amounts of cellulosic components [16].

Sugarcane bagasse (Brazil) and loblolly pine (USA) are valid models of L(h)C regarding the current prospect of biofuels such as bioethanol and biobutanol [1]. Extensive studies have been performed to meet the future challenges of bioenergy generation, because there is no self-sufficient process or technology available [16]. A pretreatment is

required as a crucial and expensive step for the bioconversion of cellulosic biomass to a more enzyme-accessible residual cellulose [1, 13].

Several types of pretreatments are explored in the literature, such as acid, alkaline, organic solvent, solid super acid, ionic liquid, ozonolysis, hydrothermal, steam explosion, microwave, microbiological, and combined methods [1]. Our biomass chemo/biotechnology group generated the technology of using very diluted thermopressurized orthophosphoric acid for the bioprocessing of sugarcane and sorghum bagasse toward biofuels [10]. Most pretreatments aim for hemicellulose and lignin solubilization or hydrolysis, thus facilitating the posterior cellulolytic enzyme's action [27]. If the solubilized sugars are oligosaccharides [e.g., xylo-oligosaccharides (XOS)] instead of free xylose and mannose, those carbohydrates are valuable products for the nutraceutical industry, with an estimated market value on the order of US $187 billion in 2010 [21].

Sugarcane bagasse hemicelluloses are composed exclusively of heteroxylan, whereas in the loblolly pine, the heteroxylan exists (38 %) but the dominant part is heteromannan (52 %). Upon partial acid hydrolysis, their products are XOS and XOS together with glucogalactomannan oligosaccharides (GGMOS) [7, 22]. They are classified as nondigestible oligosaccharides (NDOS) because the human body lacks the enzymes required to hydrolyze the β-glycosidic links. They ultimately reach the large intestine intact where they are then fermented by the appropriate microflora [7].

The biological activity of NDOSs are important for prebiotic applications because they promote the proliferation of beneficial bacteria, particularly *Lactobacillus* and *Bifidobacterium* sp., that inhibit the growth of pathogenic bacteria and reduce the risk of colon cancer owing to the formation of short-chain fatty acids (SCFAs) that prevent the evolution of mucosal aberrant crypts into tumors [3, 7, 11, 14].

Concerning the conversion of bagasse and pine L(h)C into products with added value other than as biofuel commodities and having attained the ideal kinetic conditions of the aqueous orthophosphoric acid pretreatment (e.g., heat/pressurization range; effective pH) for the generation of nutraceutical oligosaccharides, a second goal was pursued, namely, to attain probiotics.

Materials and methods

Ligno(hemi)cellulosic sources

Sugarcane bagasse (*Saccharum officinarum*) was provided by the cooperative COCAMAR (Maringá-PR-Brazil) as a courtesy of Wagner Spirandelli (CLASPAR, Curitiba-PR). Its residual sucrose was removed by extensive washing with tap water, followed once with distilled water. Loblolly pine sawdust (*Pinus taeda*) was donated by Prof. Umberto Klock and Eliana Lopes da Silva from the Laboratory of Pulp and Paper at UFPR, Curitiba-PR. Both L(h)C models were comminuted in a Waring blender and collected as mesh powders ($100 < x < 20$). The native moisture content of L(h)C or their cellulose-enriched derivatives was normalized through overnight lyophilization.

Thermopressurized pretreatment of L(h)C with orthophosphoric acid

The pretreatment with aqueous thermopressurized orthophosphoric acid (oPA) used a L(h)C to oPA solution ratio of 1:8 (12.5 %, w/v) with a progressive individual acid pH range 2.5, 2.25, 2.0, 1.75, and 1.5 (1–15 μmol/l/<0.16 %) and thermopressurization from 7 atm (171 °C) to 10 atm (185 °C) holding the manometer- and thermometer-equipped stainless steel reactor heating at the peak temperature for 2 min. No steam explosion was applied.

Bagasse and pine were always processed in parallel for any individual run and then the acid-hydro-soluble fraction was filtered using a vacuum Buchner filtration apparatus and the filtrates volume was normalized from 1:8 to 1:10 with distilled water during additional washing of the residual cellulosic biomass. Reducing and total sugars were measured using dinitrosalicylic acid (DNS) [18] and the phenol sulfuric acid [9] method, respectively.

Chromatographic analyses

Extensive thin-layer chromatographic (TLC) analyses were performed in silica gel 60 chromatoplates from Merck using acetonitrile, isopropanol, and water (15:3:5, v/v/v) as the mobile phase and orcinol–sulfuric acid at 105–110 °C as a chromogenic developing agent for soluble mono- and oligosaccharides, furfural, and hydroxymethylfurfural (HMF). The chromatoplate densitometries were performed using the software Image J 1.47v (National Institutes of Health, USA).

Monosaccharide profiles of selected samples were also confirmed by gas chromatography (GC) of the respective per-*O*-acetylated alditols in a Thermo Trace GC Ultra apparatus equipped with a FID detector and a 25-m capillary (J&W DB225) column programmed from 100 to 230 °C at 60 °C/min and then held for 12 min with He as the carrier gas at 1 ml/min.

Probiotic sources and cultivation

Two commercially available probiotics were used, *Lactobacillus casei* Shirota (Yakult®) and *Bifidobacterium animalis* (Activia®), and both were previously grown in their original suspension media after a supplementation with 0.1 g %

yeast extract and 0.1 % casamino acids and pH adjustment to 6.0. The bacterial biomasses were recovered by centrifugation (11,300 $\times g$ for 5 min), twice washed with 75 mM potassium phosphate (pH 5.0), and separately resuspended in a medium composed of 0.5 g % oPA-hydrolysed sugars (the NDOS-based carbon source from the specific pretreatment at pH 2.25/2.50; 7 atm–171 °C) either from bagasse or pine supplemented with 0.2 % each yeast extract and casamino acids with the final pH adjusted to 6.0. The cultivation under anaerobic conditions (candle jar) at 37 °C continued for 15 days with spectrophotometric monitoring of the bacterial growth at OD_{660} at given intervals. The best growth conditions were used for high-performance liquid chromatography (HPLC) on a Shimadzu LC-10 station, with a Rezex Organic Acid (ROA) column (Phenomenex) and 8 mmol/l sulfuric acid as the mobile phase at a flow rate of 0.5 ml/min and with a refraction index detector (RID).

Statistics

The Kolmogorov–Smirnov test was used to assess the normality of the distributions of the investigated parameters. Data were expressed as the mean ± standard deviation from a triplicate of each assay: $n = 180$ for preliminary tests and $n = 108$ for oPA optimization hydrolysis. For probiotics cultivation, $n = 24$. Differences were tested by a two-tailed t test, ANOVA, and Tukey's test. Pearson's correlation was used to analyze the association between parameters. The values $p < 0.05$ were considered to be statistically significant. All statistical analysis was performed using Statistica 10 software (StatSoft, Inc. Tulsa, OK, USA).

Results and discussion

The same successful technology using diluted thermopressurized orthophosphoric acid pretreatment to replace expensive enzymes [10] was again revealed to be a satisfactory catalytic tool for the conversion of starch into glucose and/or malto-syrups [12]. Under much less severe conditions, the process converted the polyfructose inulin into value-added fructo-oligosaccharides (FOS) [11] and proved to be an excellent method for the production of nutraceutical oligosaccharides from two different L(h)C sources: cane bagasse as the representative for both grasses and hardwoods and pine sawdust as the representative for softwoods.

Characterization of L(h)C products from oPA partial and total hydrolyses

For the sake of a better understanding of the present subject and its inherent difficulties, the basic compositions of the studied L(h)C models are presented in Table 1.

Table 1 Biomass composition of *Pinus taeda* and Sugarcane bagasse

	Cellulose	Hemicellulose	Lignin
Pinus taeda			
Composition [6]	45.2 % (±1.3)	21.2 % (±1.4)	27.9 % (±0.9)
Remarks	Larger fibrils [25]	Heteromannans Heteroxylans	Guaicyl units
Sugarcane bagasse			
Composition [6]	41.9 % (±3.1)	24.9 % (±1.7)	24.8 % (±5.9)
Remarks	Shorter fibrils [20]	Heteroxylans [23]	Syringyl: guaicyl

Preliminary thermopressurized phosphoric hydrolysis of L(h)C using a phytobiomass to acid solution ratio of 1:6 (w/v) and a simple glass wool filtration system with a wider pressure range of 3 atm (144 °C) to 12 atm (192 °C) revealed that each condition is able to release oligosaccharides, in different quantities and purities, except for the most severe condition (12 atm), when only free sugars and degradation products such as furfural and HMF were observed for both sources. Although these experiments had released high purity oligosaccharides, the yield, with the preliminary best production conditions, was 45 mg of oligosaccharides per gram of biomass. The optimized pressures (7–10 atm) as well the biomass to solution ratio of 1:8 (w/v) and a vacuum filtering system through sintered glass resulted in a greater nutraceutic oligosaccharides production of 90 mg/g.

As a result of the large number of experiments with the selected secondary phytobiomasses, sugarcane bagasse and pine sawdust, each overall scenario from the time course of polysaccharide hydrolysis was inspected by TLC, a fast and very useful chromatographic tool [8]. The chromogenic developing agent, hot orcinol–sulfuric acid, proved to be very sensitive (<5 μg/sample), leading to a clear color distinction among the aldohexoses (burgundy color) and aldopentoses (violet) compared to deoxysugars, furfural (blue-green), and hydroxymethylfurfural (HMF) (bright orange). The XOS standard used was obtained from native bagasse hydrolysis with trifluoroacetic acid (2 mol/l) for 30 min at 100 °C. The homologous xylosaccharide series up to $(Xyl)_7$ fitting in the linear relationship between log $[(1 - R_f)/R_f]$ versus degree of polymerization (DP) [4] has a strong correlation of $r = 0.999$ and $r^2 = 0.998$ ($p < 0.0001$), as shown in Fig. 1.

The TLC analysis showed NDOS production under all pressures assayed. The degradation product volume was increased significantly by increasing the acid strength from pH 2.5 to 1.5 and increasing the temperature and pressure (171, 176, 184 °C = 7, 8.5, 10 atm), as shown in Fig. 2. It is possible to observe a larger amount of HMF in pine

hydrolysis in the TLC compared with the bagasse under the same conditions. Other minor byproducts were also visible between R_f 0.86 (HMF) and R_f 0.66 (free xylose) in the most drastic hydrolyses. Most likely, they correspond to anhydrosugars arising from the free pentoses and hexoses. Figure 2 confirms the power and suitability of TLC to evaluate the net and comparative effects of all examined hydrolytic conditions.

The nutraceutical composition appears to be composed selectively of XOS from heteroxylans in both materials, whereas the fragmentation of the pine heteromannans is not clearly evident in the form of oligosaccharides, although in severe conditions (≥8.5 atm, pH ≤ 2.0), it was possible to observe free galactose, glucose, and mannose

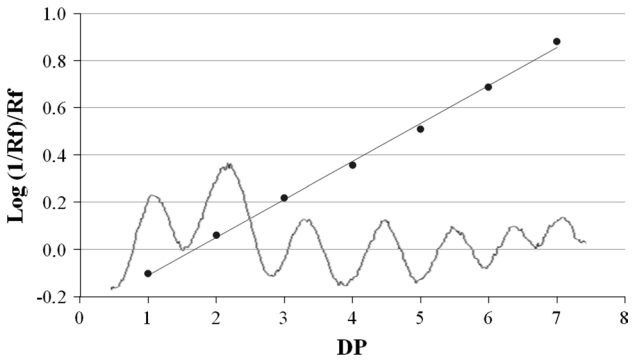

Fig. 1 Linear relationship between log $[(1 - R_f)/R_f]$ versus degree of polymerization (DP) of homologous xylosaccharide series up to $(Xyl)_7$ and a TLC densitometry graph of the XOS standard

accumulation. Under these conditions in bagasse, the release of xylose was predominant with a smaller amount of glucose, which was most likely from cellulose.

Free sugars from the productive phosphoric acid hydrolysis of bagasse and pine (10 atm/pH 1.75) were separated and quantified by per-*O*-acetylated alditols by GC as shown in Fig. 3. Because heteroxylan is a common hemicellulose for both models, the control solvolysis (using water as solvent during thermopressurization) exhibited pentoses (xylose > arabinose) in both chromatograms. A different free sugar profile arose for oPA-pretreated pine because the hexose to pentose balance (Man/Gal/Glu)/(Xyl/Ara) observed for solvolysis 19.4:73.9 evolved to 40.8:57.4 in the experiment at 10 atm/pH 1.75. Although the reducing sugars parameter remained at a level lower than that expected from hemicellulose's total hydrolysis, the monomeric sugar composition is quite representative and the progressive curves for increasing parameter severity seem to indicate that thermopressurization above the herein explored ranges would approach the quantitative recovery of the targeted hemicellulose hexoses (man > gal > glc) for pine.

The histograms shown in Fig. 4 display the total sugars released and this characterization of the time course of bagasse and pine hydrolyses with phosphoric acid from 7 atm (171 °C) to 10 atm (185 °C) comparing the pH effect. Taking into account the average values within each pressure, no statistically significant differences are observed between the L(h)C materials at 7 atm ($p = 0.4483$) and with water and pH 2.00–2.50 at 8.5 atm ($p = 0.3355$).

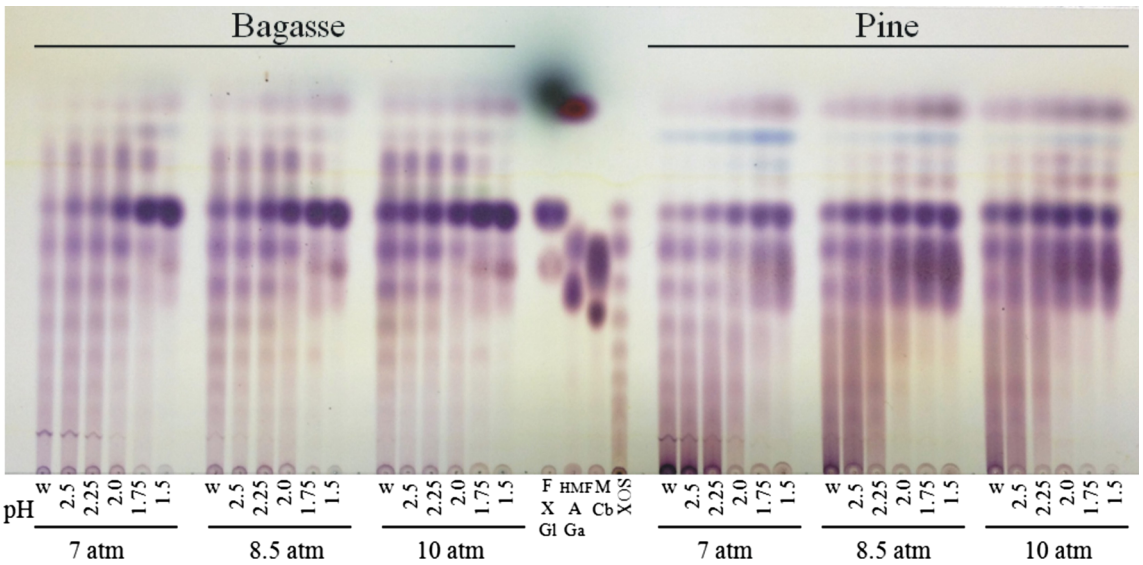

Fig. 2 TLC for the progressive hydrolytic fragmentation of whole bagasse (*left*) and pine (*right*) with increasing pressures of 7.0–10.0 (171–185 °C) and with water (*w*) and oPA pH of 1.5–2.5. *Center*: std *F* furfural, *X* xylose, *Gl* glucose, *HMF*, *A* arabinose, *Ga* galactose, *M* mannose, *Cb* cellobiose and *XOS*

Under the more severe conditions of pH 1.5–1.75 at 8.5 atm ($p = 0.0156$) and 10 atm ($p = 0.0035$), pine displayed superior release of total sugars. This result could be explained in part by the observation that once each matched hydrolysis series is finished, the resulting pH difference arising from some imbalance in the phosphoric acid solution was, on average, 0.5 pH units more acidic in the pine series. It is noteworthy that softwood afforded better results than hardwood when using microwave acid pretreatment [1]. This more acidic pH also explains the higher production of degradation products, such as HMF, and free sugars, such as glucose, galactose, and mannose in pine samples, mainly under severe conditions (≥8.5 atm, pH ≤ 2.0).

To determine the best conditions for oligosaccharide production, as shown in Table 2, we took into account the whole chromatographic profile, anticipating NDOS production of more than 70 mg/g and that these oligosaccharides represent at least 50 % of solubilized total sugars (NDOS purity). It is noteworthy that as the pressure (and temperature) increases, milder acid hydrolysis conditions or water-based solvolysis is required to optimize oligosaccharide production. In this experimental context, the best conditions found for both L(h)C residues were 7 atm/water and pH 2.25 and 2.5 and 8.5 atm/water.

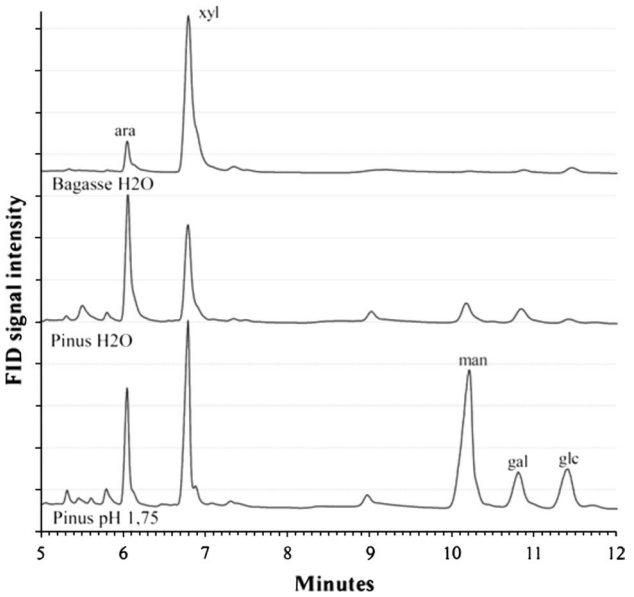

Fig. 3 Gas chromatograms of free sugar samples, pH 1.75/10 atm and the water control. Analytes as per-*O*-acetyl alditols

Probiotic growth in the mono- and oligosaccharides from phosphoric pretreatments

The probiotics cultivation was performed using NDOS mixing from the 7 atm/pH 2.25 and pH 2.5 hydrolyses whose average purities were 57.40 and 59.29 % for bagasse and pine, respectively, and were equivalent ($p = 0.2554$), allowing posterior comparison. The selection of these hydrolysates was also due to the reduced amount of degradation products, mainly anhydrides (<10 %).

The bifidobacteria and lactobacilli growth results are shown in Fig. 5. All cultivations reached the stationary phase after 15 days. Each inoculated sample's relative absorbance at 660 nm was taken as 1.0×10^7 cells/ml. During the lag phase, or the microorganism adjustment phase lasting 24 h, the free sugars (mainly xylose) were preferentially consumed. The main differential feature

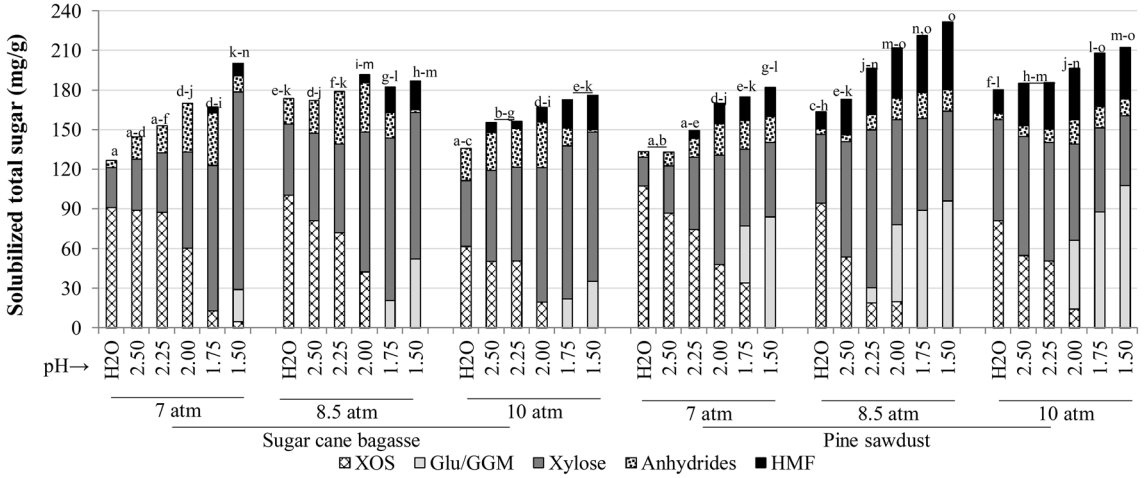

Fig. 4 Histograms of total sugars released and their qualification for oPA hydrolyses of bagasse and pine at 7–10 atm in water and at pH 2.5–1.5. (Glu/GGM) means glucose for sugarcane bagasse and the mixture of glucose, galactose, and mannose for pine sawdust. ANOVA and Tukey test showed significant differences between the pressure and pH conditions

Table 2 Optimal thermopressurization conditions for oligosaccharide release

L(h)C	Pressure (atm)	pH	Total sugars (mg/g)	Total NDOS (mg/g)	NDOS purity (%)
Bagasse	7	Water	126.61 (±3.43)^{a}	91.00 (±2.47)^{a,b}	71.87 (±2.16)^{d}
	7	2.50	144.52 (±2.59)^{b,c}	88.86 (±1.59)^{a}	61.49 (±1.84)^{a,b}
	7	2.25	152.94 (±2.86)^{c,d}	87.33 (±1.63)^{a}	57.10 (±1.71)^{a}
	8.5	Water	173.53 (±2.40)^{e}	100.26 (±1.38)^{b,c}	57.78 (±1.73)^{a}
Pine	7	Water	133.42 (±5.43)^{a,b}	107.26 (±4.37)^{c}	80.40 (±2.4 l)^{e}
	7	2.50	133.23 (±4.47)^{a,b}	86.63 (±2.90)^{a}	65.02 (±1.95)^{b}
	7	2.25	149.39 (±3.56)^{c}	74.30 (±1.77)^{d}	49.74 (±1.49)^{c}
	8.5	Water	163.44 (±9.27)^{d,e}	94.29 (±7.66)^{a,b}	57.69 (±1.73)^{a}

Tukey's test (superscript letters) shows the differences between answers ($p < 0.05$)

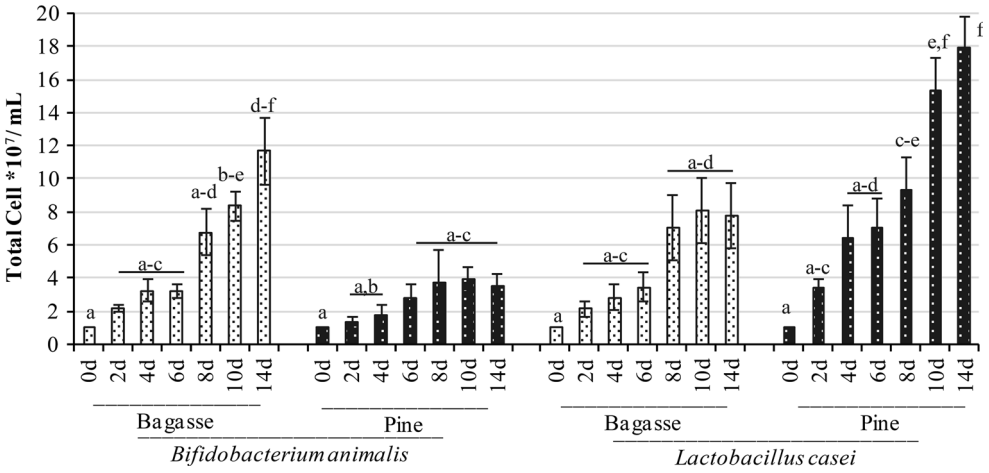

Fig. 5 Relative bacterial growth of *Lactobacillus* and *Bifidobacterium* supplemented ligno(hemi)cellulosics NDOS

between the growth of the two microorganisms was during the log phase. *Bifidobacterium* growth was observed both in bagasse ($p = 0.0008$) and pine ($p = 0.0024$), but the biomass production was three times higher with the bagasse oligosaccharides. In vivo studies with rats have shown a higher growth of this particular bacterium in animals fed with oligosaccharide diets compared with those fed with the control diet [5]. We found no difference ($p = 0.3560$) in the final pH 4.85 (±0.10) when comparing bagasse and pine NDOS fermentation.

Lactobacillus growth was observed in either bagasse ($p = 0.0041$) or pine hydrolysates ($p = 0.0001$), but bacterial biomass production was 2.25 times higher with pine oligosaccharides whose final pH was 6.58 (±0.34) compared to 4.92 (±0.01) in the case of bagasse NDOS. The colonic bacterial fermentation of oligosaccharides results in increased levels of SCFAs that stabilize an acidic pH and then prevent the pathogenic and putrefactive bacteria growth [7, 19]. The last but one pH increase may be explained by the more marked lactic acid consumption in the more advanced incubation time (>6 days; Fig. 6), which does not imply a nutraceutical power reduction because the human digestive system not only eliminates the stool within 3 days but also the intestinal bacteria. Past the 14th cultivation day, neither further growth nor absorbance decline was observed. This is in accordance with the total consumption of free sugars and NDOS from the media. Strains of *Lactobacillus* and *Bifidobacterium* are able to produce γ-aminobutyric acid (GABA) in the human intestine following a monosodium glutamate-rich alimentation. GABA is a major inhibitory neurotransmitter of the vertebrate central nervous system and it is the main inhibitory neurotransmitter in the brain, found in relatively large amounts, regulating many physiological and psychological processes. Dysfunctions in the GABA system are implicated in the sensation of pain, anxiety, and depression [2].

The probiotic growth using bagasse oligosaccharides led to no differences between bifidobacteria and lactobacilli after up to 8 days of culture. The same did not happen with the pine NDOS, which experienced a fourfold growth increase with *L. casei*. The differences in response between sources of oligosaccharides and probiotics growth made clear that the compositions of hydrolysates are not equal. Bagasse hemicellulose is composed exclusively of heteroxylans (Table 1), thus resulting just in XOS. Pine also has heteroxylans, but mainly heteromannans whose free

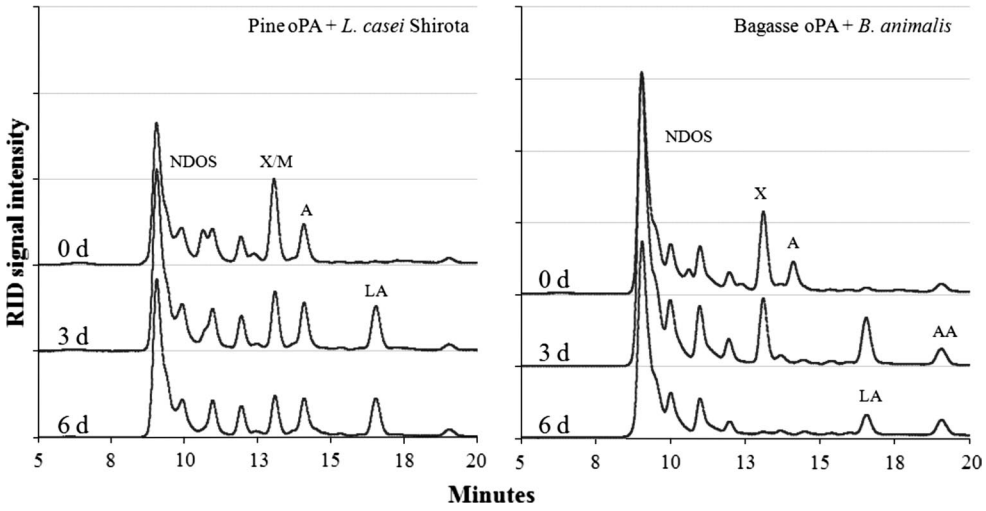

Fig. 6 High-performance liquid chromatographic profile of *Lactobacillus casei* cultivation on pine oPA and *Bifidobacterium animalis* on bagasse oPA. *NDOS* nondigestible oligosaccharides, *X* xylose, *M* mannose, *A* arabinose, *LA* acid lactic, *AA* acetic acid

mannose release was detected by TLC after only the more severe pretreatments. The probiotic cultivations revealed a synergistic effect of XOS and GGMOS, even when the second oligosaccharide source was available in small amounts. Summarizing, GGMOS had a marked positive effect on lactobacilli growth, the opposite occurring with the bifidobacteria.

The composition of the intestinal microbiota is strongly influenced by a range of factors that include dietary effects that have a marked impact on the gut environment, e.g., transit time and pH [24]. Intestinal microbiota are also involved in the pathogenesis of disease states such as inflammatory bowel disease [26]. The microbiota's capability to increase the production of SCFAs from the fermentation of carbohydrates may be indicative of gut health, because the lower pH may reduce the risk of developing gastrointestinal disorders, cancer, and cardiovascular disease. [7, 14, 15]. The production of oligosaccharides from cultivation of bifidobacteria on bagasse and those from cultivation of lactobacilli on pine were analyzed by HPLC (Fig. 6). SCFAs were detected after 3 days of culture and they were composed of lactic acid in the case of *L. casei* and lactic and acetic acid in the case of *B. animalis*. The acetic acid, as acetate, is taken up via the peripheral circulation for metabolism by the peripheral tissues and, as with lactic acid, it is able to decrease the gut pH, to increase bile salt solubility and mineral absorption, and to decrease ammonia absorption and pathogen growth [15].

Additionally, both NDOS hydrolysates from bagasse and pine, either as such or after clarification with activated charcoal (results not shown) and inoculated with the strategic prebiotic bacteria, resulted in equivalent growth rates and final bacterial densities, hence indicating the occurrence of no inhibitory effects arising from the presence of monosaccharide dehydration products (anhydrides, furfural, and HMF; soluble lignin and its fragments) as well as the benefit of the presence of phosphate (then as ammonium phosphate) as a co-nutrient source.

The whole biological activity of NDOSs is important for prebiotic applications. XOS has advantages when compared with other NDOS such as FOS because of its stability over a wide pH range. The lower values of gastric juice pH and cooking temperatures up to 100 °C [7] used in various industrial foods make XOS a good choice for these applications.

Conclusions

Cane bagasse single hemicellulosic heteroxylan hydrolysis with diluted thermopressurized phosphoric acid could be modulated by pH and temperature/pressure severity parameters to release a variety of nutraceutical XOSs (e.g., at pH 2.25 and 2.50 at 7 atm/171 °C). The same result was attained with a minor loblolly pine hemicellulose fraction because it contains a similar xylan. However, the major pine hemicellulose, heteromannan, was less prone to generate similar oligosaccharide-type fragmentation and the release of their hexose units. Mannose, glucose, and galactose required more severe conditions, leading to the formation of more degradation products, such as HMF. Within the limits of severity herein explored, the most severe (12 atm and 192 °C) led to significant loss of both released pentoses and hexoses. Overall, the free pentose recovery from bagasse and pentose plus hexose recovery of pine could be attained by increasing the severity of conditions (e.g., at pH 1.5 and 7 atm/171 °C for bagasse and the same pH and 10 atm/185 °C for pine). However, in this case, some diverting secondary reactions are steered towards the undesirable furans, whose removal would necessitate some additional post-treatment (e.g., with activated charcoal) to ensure a better yield of biofuels in the last fermentation

step. We have shown that the value-added product from pine hemicellulose partial phosphoric hydrolysis, nutraceutical oligosaccharides, was also attainable.

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