PRODUCTION OF XYLO-OLIGOSACCHARIDES BY CHEMO-ENZYMATIC TREATMENT OF AGRICULTURAL BY-PRODUCTS

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Several timbers and crop by-products were subjected to an enzymatic treatment to obtain a xylo-oligosaccharides-enriched preparation. The process was performed by means of the commercial endo-β-1,4-xylanase Buzyme 2511®. The enzymatic cocktail was applied onto the raw ground materials with yield up to 5.3 ± 1.0 g/L xylo-oligosaccharides for apple pomace. In order to make the materials more accessible to enzymatic hydrolysis, they were subjected to thermal-alkaline treatment. The biocatalysis process over the thermo-alkaline treated materials yielded xylo-oligosaccharide solutions with the following concentrations (g/l): 1.3 white poplar (*Populus alba*), 2.9 giant cane (*Arundo donax*), 3.7 apple pomace (*Malus domestica*), and 6.5 stalk of grapes (*Vitis vinifera*). The preparation resulting from biotransformation of grape stalk contained mostly xylo-oligosaccharides (96% w/v) with a small amount of xylose (3% w/v). The same ratio was obtained when pure xylan from birchwood was used as feedstock.

Keywords: Xylanase; Stalk of grape; Apple pomace; Vitis vinifera

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INTRODUCTION

Lignocellulosic biomass residues from agricultural and forestry operations are promising sources of fermentable sugars for biofuel (ethanol) production because of their high availability (Foyle et al. 2007). Cellulose is the predominant polymer in lignocellulosic biomass, with hemicellulose and lignin found in smaller amounts (Breccia et al. 1998). Several pre-treatment methods of these materials have been evaluated, usually by measuring the accessibility of cellulose to cellulases. However, techno-economical evaluations have demonstrated that future biorefineries can only become competitive if the whole range of product streams is processed (FitzPatrick et al. 2010).

Hemicelluloses, which represent between 20 and 30% of lignocellulosic biomass, consist of heterogeneous polysaccharides found in association with cellulose and lignin. Xylans, the main constituents of hemicelluloses, are heteropolysaccharides that are soluble in alkaline solutions (Breccia et al. 1995). Their chemical structure comprises a homopolymeric backbone chain of 1,4-linked L-D-xylopyranose units with branched substituents of O-acetyl, α-L-arabinofuranosyl, α-1,2-linked glucuronic, or 4-O-methyl-glucuronic acid (Joseleau et al. 1992). Not only can hemicellulose be utilized for ethanol production, but also it can serve as a source of other high value-added chemicals, with wide applications in the food, feed, pharmaceutical, and cosmetics industries. Some

examples include xylitol, 2,3-butanediol, lactic, itaconic, and ferulic acids (for a review see Peng et al. 2011). Xylo-oligosaccharides (XOS) are emerging as non-digestible but fermentable carbohydrates that present prebiotic properties (Rurangwa et al. 2009). Both XOS and fructo-oligosaccharides (FOS) were associated with the reduction of aberrant crypt foci in the colon of rats and, in some cases, XOS showed a greater effect on the stimulus of beneficial bacterial population in comparison with FOS (Hsu et al. 2004).

The production of xylo-oligosaccharides can be attained by means of endo-β-1,4-xylanases. Buzyme 2511® is a formulated product containing such activity, mostly employed as a pre-bleaching treatment for pulp in the paper industry. For that reason, it is available with a relatively low cost for fine chemicals production. This works aims at: a) the characterization of the enzymatic cocktail Buzyme 2511® in terms of its activity with different substrates, and b) the preparation of XOS from agricultural by-products by the application of this cocktail.

EXPERIMENTAL

Materials

The chemicals p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-α-L-rhamno-pyranoside, p-nitrophenyl-α-D-mannopyranoside, p-nitrofenil-β-D-xylopyranoside, p-nitrophenyl-β-D-galactopyranoside, birchwood xylan, apple pectin, and low viscosity carboxymethylcellulose were purchased from Sigma-Aldrich (USA). Microcrystalline cellulose was obtained from Parafarm (Argentina). The xylanase preparation Buzyme 2511® was obtained from Buckman Laboratories International, Inc. All other chemicals were from standard sources.

Lignocellulose Feedstocks

The following materials were used as lignocellulose sources: wood chips of white poplar (*Populus alba*) and giant cane (*Arundo donax*), marc and stalk of grape (*Vitis vinifera*), and pomace of apple (*Malus domestica*). The materials were dried for 48 h at 105°C, mill grounded, and suspended (50 g/L) in 50 mM phosphate buffer (pH 6.0) for enzymatic hydrolysis. When indicated in text, the mince timbers were treated thermo-chemically by suspending them in sodium hydroxide (0.1 M NaOH) and heated for 1 h at 121°C. The samples were neutralized with HCl up to pH 6.8 to 7.

Enzymatic Assays

Depolymerizing enzymes were assayed by measuring the amount of reducing sugars released from different polysaccharides. The xylanase preparation Buzyme 2511® was appropriately diluted (50 µL) and incubated with 450 µL of 10 g/L substrate suspension for 30 min at 50°C. The reaction was stopped by adding 500 µL of 3,5-di-nitrosalicylic acid (DNS) and heated for 10 min at 100°C. Samples were allowed to cool, and absorbance was read at 540 nm (Miller 1959). One unit of the enzyme was defined as the amount of enzyme able to release 1 µmol of reducing sugars (considering the main component monomer of each substrate) per minute.

Exo-acting glycosidase activities were quantified using 5 µL of the corresponding substrate (70 mM p-nitrophenyl-derivative in dimethylformamide), 895 µL of 50 mM phosphate buffer (pH 7.0), and 100 µL of diluted enzyme. The reaction occurred for 30 min at 39°C and was stopped by adding 100 µL of 0.1 M NaOH. Absorbance was measured at 420 nm, and the amount of p-nitrophenol released was calculated using its extinction coefficient (ε_{420} nm = 1.6 x 104 M^{-1} cm^{-1}) (Orrillo et al. 2007). One enzyme unit was defined as the amount of enzyme that released 1 µmol of p-nitrophenol in 1 min at the indicated temperature. For reactions at different pH values the following buffers (50 mM) were used: sodium citrate (pH: 4.5-5), sodium phosphate (pH: 6-8), and sodium carbonate (pH: 9-10).

Analytical Assays

The products of the enzymatic reaction were analyzed by loading known volumes onto a thin layer chromatography plate (Silicagel 60 W). The chromatography was performed using ethyl-acetate/2-propanol/water (3:2:2) as the mobile phase, and the plate was stained with anthrone reagent. The 32-bit color images were split into red, green, and blue (RGB) components using the software ImageJ 1.38x (National Institutes of Health, USA). Images corresponding to the red component were chosen due to the highest signal to noise ratio. Next, integrated optical density units were used for quantification of sugars (xylose and xylo-oligosaccharides) using xylose as a standard. Protein concentration was determined by Bradford's method (1976) using egg white lysozyme as a standard. The hemicellulose content was estimated according to Breccia et al. (1995).

RESULTS AND DISCUSSION

Enzymatic Hydrolysis of Timbers and Crop By-products

Different crop by-products and timbers were chosen with the goal of transforming them into highly valuable chemicals. Typical raw materials reported for XOS production are hardwoods, corncobs, straws, bagasse, hulls, malt cakes, and bran (Vázquez et al. 2000). In the present work white poplar, giant cane, grape marc, apple pomace, and grape stalk were selected because of their low cost, which is due to the fact that they are abundant agricultural by-products in Argentina. Their hemicellulose content is shown in Table 1.

Table 1. Hemicellulose Content (in a dry basis) of Selected Agricultural By-products

Source	Hemicellulose (%w/w)
White poplar chips	20.5 ± 0.2
Giant cane chips	30.8 ± 0.4
Grape marc	11.7 ± 0.8
Apple pomace	9.7 ± 2.2
Grape stalk	19.6 ± 1.5

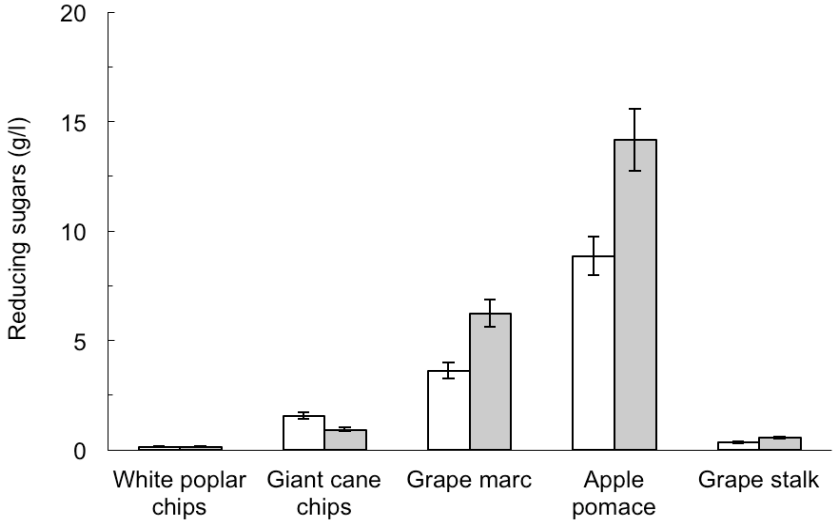

Figure 1. Hydrolysis of raw lignocellulosic feedstocks by enzymatic treatment with endo-xylanase preparation (g/L reducing sugars using xylose as standard). (□) Starting material, (■) after enzymatic treatment (pH 6, 30°C, 27 h)

Enzymatic hydrolysis of the milled lignocellulosic materials was performed for 27 h at 30°C (Fig. 1). Apple pomace and grape marc were the only two materials that showed significant enzymatic depolymerization, with 5.3 ± 1.0 and 2.6 ± 0.5 g/L of reducing sugars being gained during the enzymatic treatment, respectively. Remarkably, both substrates undergo a yeast fermentation process during manufacture of their main product (wine or cider) (Joshi and Sandhu, 1996; Spigno et al. 2008). This data suggests that the fermentation process makes the vegetal tissues more accessible to enzymatic degradation and recalled the need for a pre-treatment of the materials. The hydrolysis of the selected materials by simple enzymatic treatment signaled at least two potential sources of xylo-oligosaccharides.

Characterization of the Xylanase Preparation

The xylanase preparation Buzyme 2511® was characterized in order to set up the physical-chemical parameters of the lignocellulosic materials' biotransformation. The preparation was found to be active (>50%) in the pH range 5 to 7, with an optimum at pH 6 (Fig. 2). The apparent optimum temperature was observed at 50°C, and 40% of the activity was displayed at room temperature (Fig. 2). These values are in agreement with the recently purified endo-xylanase of Buzyme 2511® preparation (Tabosa-Vaz et al. 2011).

Subsequently, the preparation was explored for several glycoside-hydrolase activities. The substrates were chosen by taking into account the chemical composition of the lignocellulosic materials. The purified polysaccharides birchwood xylan, apple pectin, low viscosity carboxymethylcellulose, and microcrystalline cellulose were used to test endo-glycosidases activities (endo-β-1,4-xylanase, pectinase, cellulase, endo-β-1,4-glucanase, respectively) (Fig. 3A).

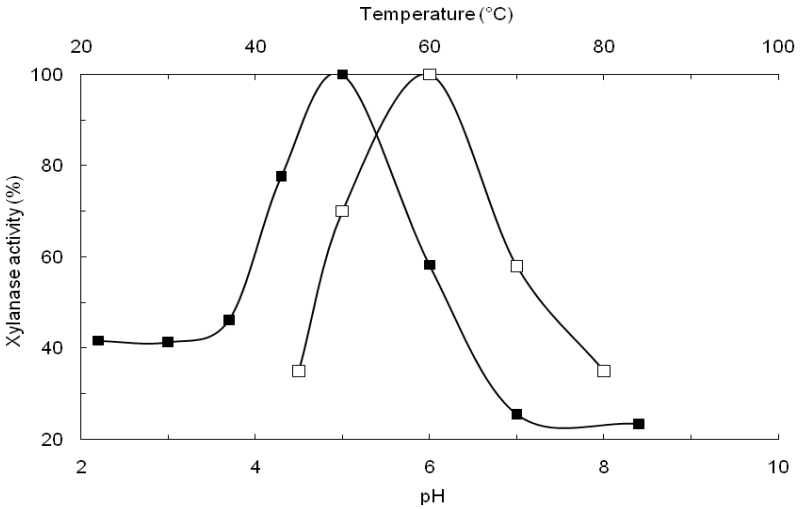

Figure 2. Optimum pH (□) and temperature (■) of the endo-xylanase activity of Buzyme 2511® preparation. One hundred percent activity corresponded to 1.5 U/mL.

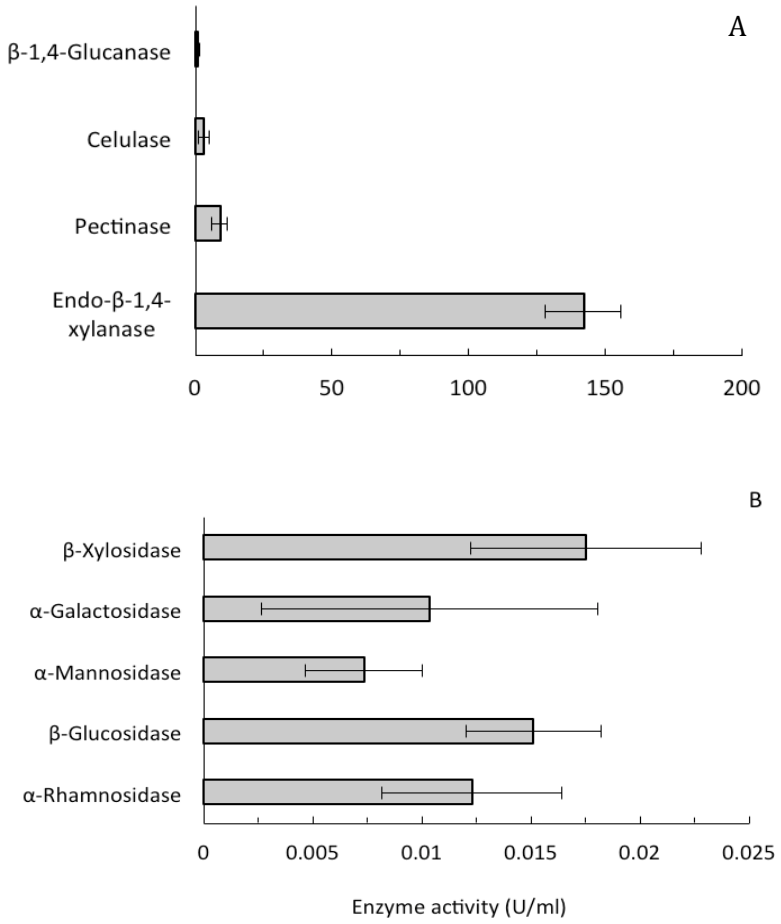

Figure 3. Glycosyl hydrolases detected in the commercial preparation Buzyme 2511®. A: endoglycosidases, B: exoglycosidases

The highest activity was achieved when birchwood xylan was used as the substrate (142 ± 14 U/mL), while the rest of the polysaccharides were also hydrolyzed, but to a lesser extent (less than 10% relative activity). On the other hand, exo-acting activities (α-mannosidase, α-rhamnosidase, β-glucosidase, β-xylosidase, and β-galactosidase activities) were detected using p-nitrophenyl-derivatives of the corresponding monosaccharides (Fig. 3B). Exo-type glycosidases were minor as judged by the several orders of magnitude between them and endo-acting glycosidases.

In sum, the major activity was the endo-type xylanase, while the exo-glycosidases detected are likely to act synergistically with the depolymerizing activities. The ratio between monomeric and oligomeric end products will depend on the balance among the enzymatic activities of the preparation. Most reported endo-xylanases produce XOS of low polymerization degree (DP2 to DP5 xylose residues) as main reaction products (Breccia et al. 1998). Since a high concentration of exo-type glycosidases would be detrimental for XOS yield, the identification of the component activities of the preparation will determine its suitability for XOS production.

Time Course of Xylo-Oligosaccharide Production

A bench-scale reaction was carried out in order to obtain a XOS-rich preparation. The pH value was adjusted to 6.0 and temperature was set at 50°C, taking into account the characteristics of the enzymatic preparation Buzyme 2511®. Apple pomace was used as the substrate because of the highest hydrolysis detected among the tested lignocellulosic materials. The kinetics of hydrolysis were evaluated by following the reducing sugars liberated from the substrate. XOS production was found to be linear up to 6 h, and then the curve reached a plateau up to 20 h (Fig. 4). The profile of oligosaccharide production was similar to that reported for a crude enzymatic preparation from *Bacillus licheniformis* (Pérez et al. 2007). After 20 h of reaction, the concentration of reducing sugars diminished. This drop could be attributed to microbial growth, which is a usual problem among industrial biotrans-formations (Solle et al. 2004). The net gain of reducing sugars was ca. 3.5 g/L, i.e. 6% yield with respect to the starting raw material. Although insoluble substrates may limit the enzymatic reactions (Solle et al. 2004), the yield of the process performed under agitation did not improve, compared to the non-agitated reactor (Fig. 4). Apple pomace is a bulky waste product often considered to be of little value as animal foodstuff or for pectin production (Stredansky and Conti 1999). Xylo-oligosaccharide production may be an alternative utilization of this by-product.

Thermal-alkaline Treatment of the Lignocellulosic Materials and Enzymatic Hydrolysis

Several strategies have been performed to hydrolyze lignocellulosic materials with the aim of obtaining highly valuable products. Either chemical or enzymatic de-polymerization techniques or a combination of both underlie the vast majority. Recently, vom Stein et al. (2010) reported a chemical method that mimics the active site of most glycoside hydrolases by using dicarboxylic acids. This approach was shown to efficiently de-polymerize cellulose at relatively mild temperatures (100 to 125 °C) and high ionic strengths.

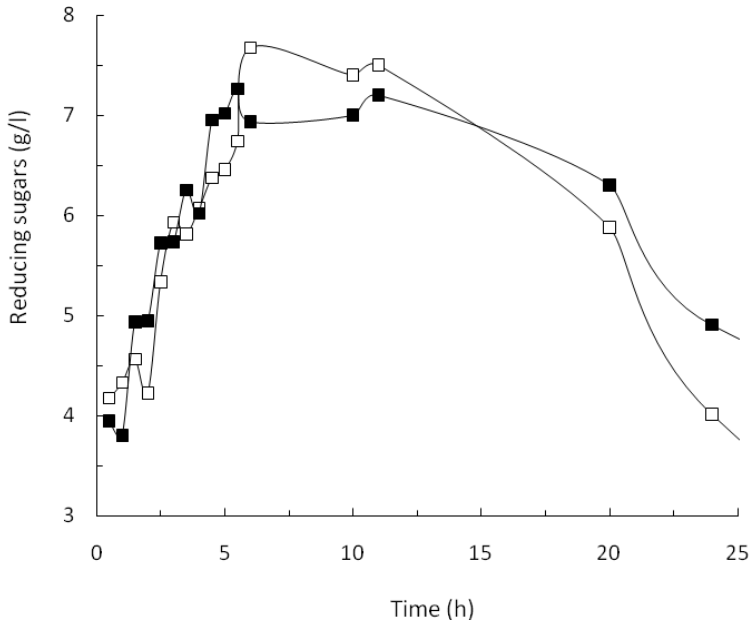

Figure 4. Time course of endo-xylanase reaction using apple pomace as substrate in non-agitated reactor (■) and agitated reactor (□)

For xylan de-polymerization, several authors have noted the need for a thermal-alkaline treatment before the biotransformation (Dien et al. 2006; Selig et al. 2009; Wan et al. 2011). The materials were subjected to pre-treatment by adding sodium hydroxide (0.1 M NaOH) and heating at 121°C for 1 hour. The mixture was neutralized with hydrochloric acid, and the pH value was adjusted to 6.0 prior to enzymatic hydrolysis. Mirahmadi et al. (2010) grouped the processes of sodium hydroxide pre-treatment of timbers in those performed with high concentration of NaOH (typically, 7-20%w/v) at room temperature and those that employ low concentration of NaOH at high temperature. The process performed in this work belongs to the second group with NaOH concentration of 0.4%w/v at 121°C. This choice would lower the amount of waste in comparison with the "high concentration" processes, being more environmentally friendly. On the other hand, the energy cost will be higher because of the temperature required. Although the process alternatives should undergo a techno-economical evaluation, this is outside of the scope of this particular study (Sassner et al. 2008). The alkaline pre-treatment aims at the selection of the suitable feedstock for xylo-oligosaccharide production.

The biotransformation process of the thermal-alkaline-treated materials produced significant increments of XOS for white poplar and giant cane chips, and grape stalk in comparison with the non-pretreated materials (Fig. 5 and Fig. 1, respectively). The difference between the integrated areas, excluding that of the monomer, represents the XOS production (Debiere et al. 1990). The application of alkaline solutions can remove or modify the lignin barrier, by disrupting the structural linkages between xylan and lignin, with a consequent increase in the porosity of the biomass (Xu et al. 2010). Since lignin is more abundant in woody tissues, it is not surprising that white poplar and giant

cane chips, and grape stalk recalled the need for NaOH treatment. On the other hand, grape marc and apple pomace could be used as raw materials – as demonstrated before, Fig. 1 – in part for their non-woody character and in part because those materials have been fermented during the manufacturing of the main industrial product.

Grape stalk gave the highest yield (6.5 g/L) (Fig. 5), which was similar to that obtained from corncobs (8.5 g/l XOS) using a thermostable xylanase from *Paecilomyces thermophila* at 70°C (Teng et al. 2010). For that reason, grape stalk was selected for further experiments, in spite of the fact that its hemicellulose content is the lowest among the woody materials.

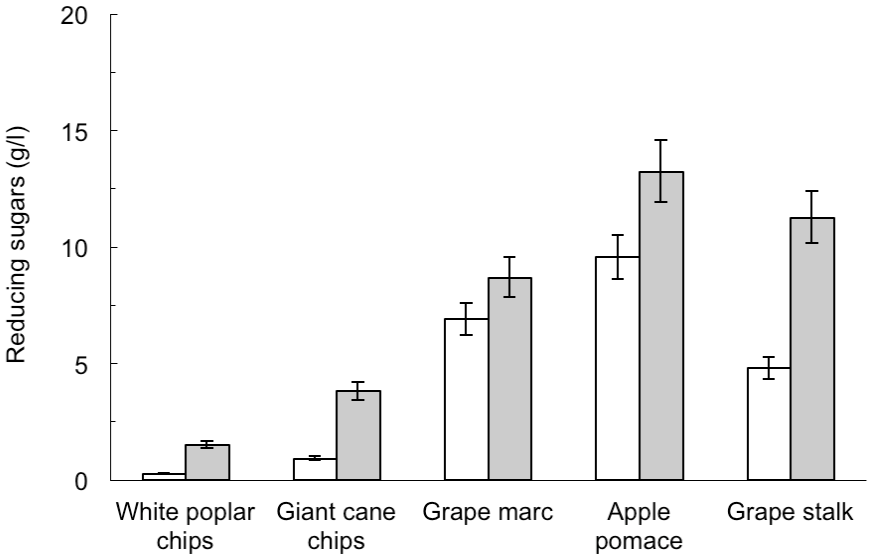

Figure 5. Enzymatic hydrolysis of lignocellulosic feedstocks after thermal-alkaline pretreatment (0.1M NaOH-1h-121°C) Results are expressed as g/l reducing sugars using xylose as standard. Starting material (□), after enzymatic treatment (■) (pH 6, 50°C, 6 h).

The biocatalytic process (pH 6, 50°C, 6 h) was performed with the hemicellulose-rich fraction of grape stalks and commercial birchwood xylan as substrates (Table 2 – Fig. 6). The monomer xylose represented only 3 and 3.5% of total products generated for both birchwood xylan and grape stalk hemicellulose, respectively.

Table 2. Quantification of Reaction Products from Xylanase Buzyme 2511® Acting on Commercial Birchwood Xylan and Hemicellulose-Rich Fraction of Grape Stalks

Substrate	Xylose (g/l)	Xylo-oligosaccharides (g/L)
Birchwood xylan	0.65 ± 0.05	17.8 ± 0.3
Birchwood xylan*	ND	ND
Grape stalk hemicellulose	0.15 ± 0.02	4.8 ± 0.1
Grape stalk hemicelluloses*	ND	ND

The integration of the spots density was expressed as g/L of xylose equivalents. ND: not detected; E: enzyme. * Samples using inactivated enzyme (10 min – 100°C).

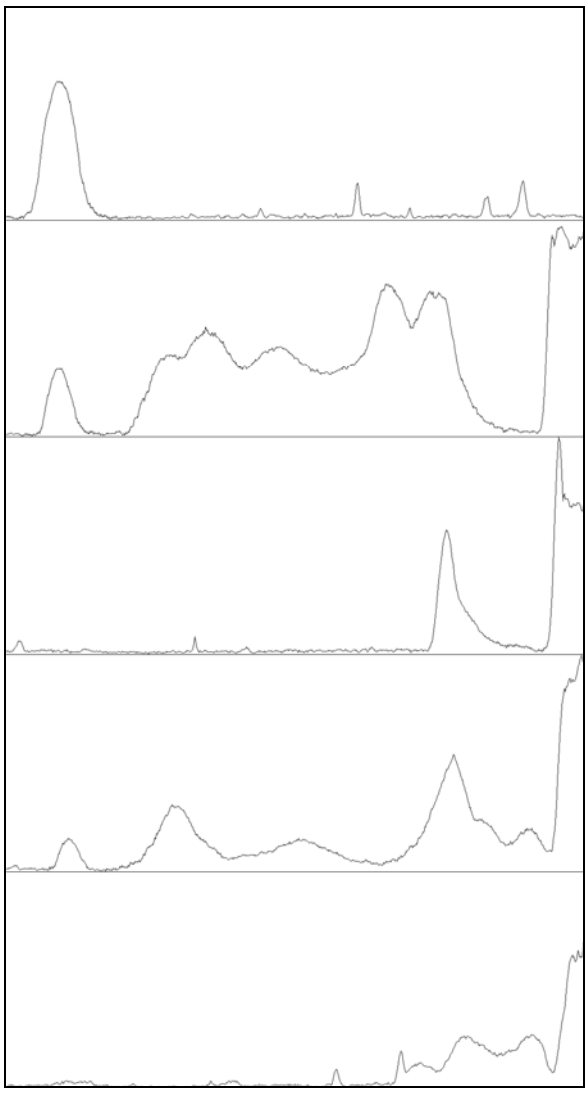

Figure 6. Thin layer chromatography densitograms of the enzymatic reaction products using birchwood xylan and grapes stalk as substrates (pH 6, 50°C, 6 h).

The pattern of hydrolysis is congruent with the endo-mode of action of the xylanase preparation. The same behavior was recently demonstrated for the *Penicillium funiculosum* GH10 xylanase D, which was able to release mostly xylo-oligosaccharides and xylobiose from xylans (Lafond et al. 2011). The xylanase Buzyme 2511® produced 17.8 g/L of XOS using pure xylan from birchwood and 4.8 g/l XOS using pre-treated grape stalk (Table 2). The thermal-alkaline treatment of grape stalk produced a significant increment of low molecular weight sugars other than xylose (ca. 4 g/L) and other unidentified colored components (Fig. 5). Such compounds may cause enzyme inhibition, which in fact affects the yield of the enzymatic process. Steam explosion treatment of lignocellulosic materials provided faster (5 min) accessibility of the biopolymers for hydrolytic enzymes, but the production of undesired fermentable monosaccharides was even higher (average 18 g/L xylose) (Teng et al. 2010).

CONCLUSIONS

1. The commercial xylanase preparation Buzyme 2511® was found to have endo-type β-1,4-xylanase as the major depolymerizing activity, while several exo-glycosidase activities (α-mannosidase, α-rhamnosidase, β-glucosidase, β-xylosidase, and β-galactosidase) were detected in minor amounts.
2. The xylanase preparation was able to hydrolyze agricultural by-products, rendering an oligosaccharides-rich mixture at mild conditions (50ºC and pH 6). The highest yield was obtained with grape stalk after a thermo-chemical treatment, although this study demonstrated that XOS-enriched solution could also be prepared from a raw material like apple pomace without the need of such pretreatment.

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REFERENCES CITED

Breccia, J. D., Castro, G. R., and Siñeriz, F. (1995). "Screening of xylanolytic bacteria using a colour plate method," *J. Appl. Bacteriol.* 78, 469-472.

Breccia, J. D., Torto, N., Gorton, L., Siñeriz, F., and Hatti-Kaul R. (1998). "Specificity and mode of action of a thermostable xylanase from *Bacillus amyloliquefaciens*. On-line monitoring of hydrolysis products," *Appl. Biochem. Biotechnol.* 69, 31-40.

Debiere, P., Priem, B., Strecker, G., and Vignon, M. (1990). "Purification and properties of an endo-1,4-xylanase excreted by a hydrolytic thermophilic anaerobe, *Clostridium thermolacticum*. A proposal for its action mechanism on larchwood 4-O-methylglucuronoxylan," *J. Biochem.* 187, 573-580.

Dien, B. S., Li, X., Iten, L. B., Jordan, D. B., Nichols, N. N., O'Bryan, J. P., and Cotta, M. A. (2006). "Enzymatic saccharification of hot-water pretreated corn fiber for production of monosaccharides," *Enz. Microb. Technol.* 39, 1137-1144.

Foyle, T., Jennings, L., and Mulcahy, P. (2007). "Compositional analysis of lignocellulosic materials: Evaluation of methods used for sugar analysis of waste paper and straw," *Bioresource Technol.* 98, 3026-3036.

FitzPatrick, M., Champagne, P., Cunningham, M. F., and Whitney, R. A. (2010). "A biorefinery processing perspective: Treatment of lignocellulosic materials for the production of value-added products," *Bioresource Technol.* 101, 8915-8922.

Hsu, C., Liao, J., Chung,Y., Hsieh, C., and Chan, Y. (2004). "Xylooligosaccharides and fructooligosaccharides affect the intestinal microbiota and precancerous colonic lesion development in rats," *J. Nutr.* 134, 1523-1528.

Joseleau, J. P., Comptat, J., and Ruel, K. (1992). "Chemical structure of xylans and their interaction in the plant cell walls," *Xylans and Xylanases*, Visser, J., Beldman, G.,

Kusters-Van Someren, M. A., and Voragen, A. G. J. (eds.), Elsevier Science, Amsterdam, 1- 15.

Joshi, V. K., and Sandhu, D. K. (1996). "Preparation and evaluation of an animal feed byproduct produced by solid-state fermentation of apple pomace," *Bioresource Technol.* 56, 251-255.

Lafond, M., Tauzin, A., Desseaux, V., Bonnin, E., Ajandouz, E., and Giardina, T. (2011). "GH10 xylanase D from *Penicillium funiculosum*: Biochemical studies and xylooligosaccharide Production," *Microb. Cell Factories*, 10:20.

Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugars," *Anal. Chem.* 31, 426-428.

Mirahmadi, K., Mohseni Kabir, M., Jeihanipour, A., Karimi, K., and Taherzadeh, M. J. (2010). "Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production," *BioResources* 5, 928-938.

Orrillo, A. G., Ledesma, P., Delgado, O. D., Spagna, G., and Breccia, J. D. (2007). "Cold-active α-L-rhamnosidase from psychrotolerant bacteria isolated from a sub-Antarctic ecosystem," *Enz. Microb. Technol.* 40, 236-241.

Peng, F., Ren, J. L., Xu, F., and Sun, R.-C. (2011). "Chemicals from hemicelluloses: A review," *Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass,* Zhu, J. Y., Zhang, X., and Pan, X. (eds.), American Chemical Society, USA, 219-259.

Pérez, M., Piad, R., Milian, G., Felipe, M. G., Ferreira, A., Mancilha, I. M., Laurencio, M., and Almeida e Silva, J. B. (2007). "Preparation of a crude enzymatic from *Bacillus licheniformes* E-44 and its evaluation in the hydrolysis of *Saccharomyces cerevisiae* cell walls," *Enz. Microb. Technol.* 40, 452-455.

Roberfroid, M. B. (2005). "Introducing inulin-type fructans," *British J. Nutr.* 93, 13-25.

Rurangwa, E., Laranja, J. L., Van Houdt, R., Delaedt, Y., Geraylou, Z., Van de Wiele, T., Van Loo, T., Van Craeyveld, V., Courtin, C. M., Delcour, J. A., and Ollevier, F. (2009). "Selected nondigestible carbohydrates and prebiotics support the growth of probiotic fish bacteria mono-cultures in vitro," *J. Appl. Microbiol.* 106, 932-940.

Sassner, P., Galbe, M., and Zacchi G. (2008) "Techno-economic evaluation of bioethanol production from three different lignocellulosic materials," *Biomass Bioenergy* 32, 422-430.

Selig, M.,Vinzant, T., Himmel, M., and Decker, S. (2009). "The effect of lignin removal by alkaline peroxide pretreatment on the susceptibility of corn stover to purified cellulolytic and xylanolytic enzymes," *App. Biochem. Biotechnol.,* 155, 94-103.

Solle, D., Faurie, R., Breccia, J., Scheper, T., and Hitzmann, B. (2004). "Modelling of an industrial biotransformation process for tryptophan production," Proc. 9th International Symposium on Computer Applications in Biotechnology, 28-31.

Spigno, G., Pizzorno T., and De Faveri, D. M. (2008). "Cellulose and hemicelluloses recovery from grape stalks," *Bioresource Technol.* 99, 4329-4337.

Stredansky, M., and Conti, E. (1999). "Xanthan production by solid state fermentation," *Process Biochem.* 34, 581-587.

Tabosa-Vaz, S., Kumar, A., Permaul, K., and Singh, S. (2011). "Purification and biochemical characterization of a xylanase purified from a crude enzyme extract for the determination of active site residues," *African J. Biochem. Res*. 5, 43-56.

Teng, C., Qiaojuan, Y., Zhengqiang, J., Guangsen, F., and Bo, S. (2010) "Production of xylooligosaccharides from the steam explosion liquor of corncobs coupled with enzymatic hydrolysis using a thermostable xylanase," *Bioresource Technol.* 101, 7679-7682.

Vázquez, M. J., Alonso, J. L., Domínguez, H., and Parajó, J. C. (2000). "Xylooligosaccharides: manufacture and applications," *Trends Food Sci. Technol.* 11, 387-393.

vom Stein, T., Grande, P., Sibilla, F. Commandeur, U., Fischer, R., Leitnera, W., and Domínguez de María, P. (2010). "Salt-assisted organic-acid-catalyzed depolymerization of cellulose," *Green Chem.* 12, 1844-1849.

Wan, C., Zhou, Y., and Li, Y. (2011). "Liquid hot water and alkaline pretreatment of soybean straw for improving cellulose digestibility," *Bioresource Technol.* 102, 6254-6259.

Xu, J., Cheng, J. J., Sharma-Shivappa, R. R., and Burns, J. C. (2010). "Sodium hydroxide pretreatment of switchgrass for ethanol production," *Energy fuels* 24, 2113-2119.

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