XYLOOLIGOSACCHARIDES PRODUCTION FROM CORN STRAW USING ENDOXYLANASE OF *Thermomyces lanuginosus* PC7S1T

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ABSTRACT

This study investigates the production of xylooligosaccharides (XOS) from corn straw hemicellulose using endoxylanase from *Thermomyces lanuginosus* PC7S1T. Corn straw, an abundant agricultural residue in Brazil, was alkali pretreated to extract hemicellulose, which was subsequently hydrolyzed by purified endoxylanase. The hydrolysis produced XOS, primarily xylobiose, xylotriose, and xylotetraose, with a concentration of 4.44 g/L for beechwood xylan and 3.27 g/L for corn straw hemicellulose. The enzyme's efficiency was highlighted by its ability to produce XOS without significant production of xylose, suggesting a targeted hydrolysis of the xylan chain. Notably, the production of XOS was not significantly influenced by reaction time, indicating that the enzyme can achieve high yields in shorter periods, enhancing its industriais applicability. The study demonstrates the potential of utilizing corn straw hemicellulose as a sustainable substrate for XOS production, with further optimization of the hydrolysis process expected to improve yields. These results emphasize the potential of *Thermomyces lanuginosus* PC7S1Tendoxylanase for converting lignocellulosic residues into valuable prebiotic compounds, thereby facilitating the development of sustainable bioprocesses.
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Keywords: Agro-industrial waste. Fungal enzyme. Prebiotic. Biotechnological application.

1 INTRODUCTION

The utilization of lignocellulosic materials is represented by the innovative and sustainable process of producing xylooligosaccharides (XOS) from agro-industrial residues, such as corn straw. The action of fungal xylanases can convert these residues, which are abundant in hemicellulose, particularly xylan, into XOS. The 1,4-β-D-glycosidic bonds of the xylan main chain can be hydrolyzed by enzymes known as xylanases (EC 3.2.1.8) to generate minor chains of xylooligosaccharides[1].

The primary objective of this investigation is to examine the utilization of corn straw, an abundant agricultural residue in Brazil, as a substrate for the production of xylooligosaccharides (XOS). These XOS are prebiotic compounds that are composed of 2-7 xylose molecules that are connected by β-linkages. The specific type of XOS, including xylobiose, xylotriose, xylotetraose, and xylopentaose, is determined by the number of xylose molecules in the chain[2]. These oligosaccharides have been the subject of extensive research due to their beneficial properties for human health, which include the reduction of serum glucose and lipid levels[3], and consequently, obesity[4]. Therefore, the purpose of this study was to evaluate the production of XOS from hemicellulose derived from corn straw by utilizing endoxylanase from the filamentous fungus *Thermomyces lanuginosus* PC7S1T isolated from the Atlantic forest of western Paraná.

2 MATERIAL & METHODS

Pretreatment of corn straw for hemicellulose extraction

The corn straw was obtained from local agricultural fields in Cascavel, Paraná state, Brazil. It was ground in a blender and treated with NaOH 2% stirring in a magnetic stirrer at 25 ºC for 2 hours. The mixture of corn straw and NaOH was kept overnight in a drying oven at 80 °C to reduce the total volume. The final content was filtered, the pH adjusted to 7.0, and three volumes of 96% ethanol were added and kept at 8 °C for 24 h to precipitate the hemicellulose. The hemicellulose fraction was obtained after centrifugation at 4000 rpm for 10 min and dehydrated in a drying oven at 50 °C according to methodology of Nascimento *et al*, 2022[5].

Cultivation and purification of xylanase

The fungus *Thermomyces lanuginosus* PC7S1T isolated from the Atlantic forest of western Paraná. It was cultivated in Erlenmeyer flasks with 25 mL of Czapek mineral medium supplemented with 1% corn straw and incubated at 42 °C for 4 days in order to produce xylanase. The crude extract was obtained through vacuum filtration and dialyzed for 18 hours. The extracellular crude extract was loaded onto a DEAE-Sephadex chromatography column and eluted with a linear NaCl gradient

for purification. Pure endoxylanase was obtained by loading the concentrated sample onto a Sephadex G-75 column following DEAE.

Hydrolysis of corn straw hemicellulose with endoxylanase

Hydrolysis was carried out with 1% (w/v) hemicellulose obtained from corn straw, dissolved in 100 mM sodium phosphate buffer, pH 6.5, with the addition of an equivalent of 50 units of purified endoxylanase from *Thermomyces lanuginosus*. This mixture was incubated at 65 °C under stirring at 150 rpm for until 48 h. Aliquots were collected at 1h, 2h, 4h, 6h, 8h, 12h, 24h, 36h and 48h, the reaction was stopped in a boiling bath for 10 min, the supernatant was collected after centrifugation for measurement of the XOS using the Miller method[6].

Analysis of hydrolysis products by thin layer chromatography (TLC)

The samples of the hydrolysis products of beechwood xylan and corn straw hemicelulose were spotted on silica plates according to Della Torre *et al*.[7], the solvent system consisted of butanol:ethanol:water (5:3:2, v/v/v) and the hydrolysis products were detected by spraying with 0.2% (w/v) orcinol in methanol:sulphuric acid (9:1, v/v) followed by heating at 100 ºC for a few minutes. Xylose (X1) xylobiose(X2) xylotriose (X3) and xylotetraose(X4) were used as standard.

3 RESULTS & DISCUSSION

The hydrolysis using endoxylanase from *Thermomyces lanuginosus* on xylan from beechwood and hemicellulose obtained from corn straw corn straw yielded 4.44 and 3.27 g/L of XOS, respectively. Additionally, the structural distinction between beechwood xylan and corn straw likely contributed to the lower production of XOS. The production of XOS using these substrates was greater as the reaction time increased, figure 1a shows that in 1 hour of reaction only 2.52 g/L of XOS was produced, while at the end of 48 hours it was possible to obtain 4.44 g/L of the prebiotic. The enzymatic hydrolysis process of *Thermomyces* endoxylanase with hemicellulose obtained from corn straw can still be optimized. This is evidenced by the reported increase in XOS production from the hydrolysis of pretreated corn cob using endoxylanase from *A. oryzae* MTCC 5154, which produced 10.2 g/L after optimization using 1.5 g of substrate for 300 U of enzyme[8].

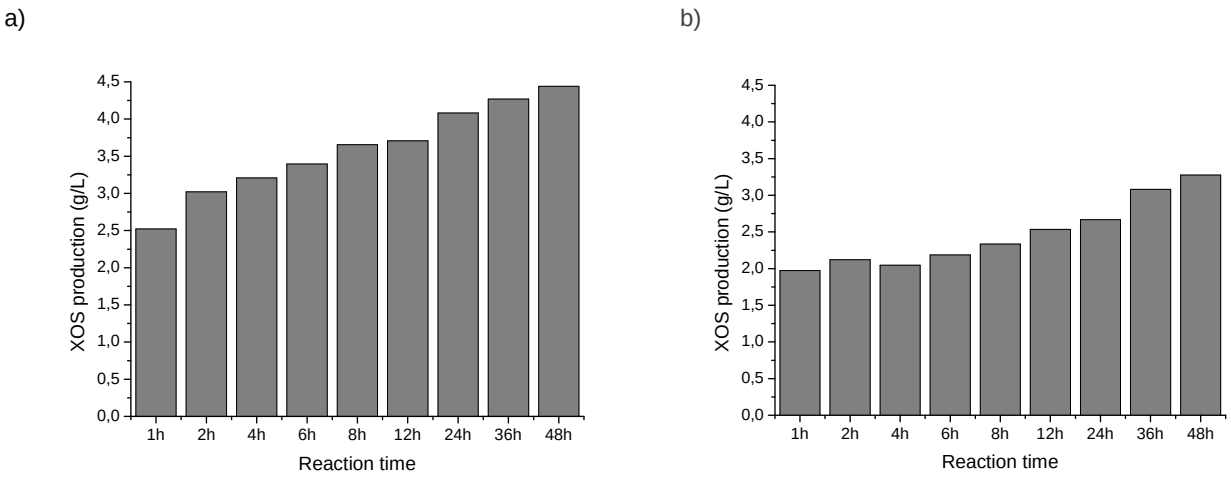

Figure 1 XOS production from beechwood xylan 1% (a) and corn straw hemicellulose (b) using endoxylanase of *Thermomyces lanuginosus* PC7S1T.

The identification of xylooligosaccharides present in the hydrolysis products of corn straw hemicelluloses was verified using TLC (Figure 2). The xylobiose, xylotriose and xylotetraose were the predominant products of hydrolysis by endoxylanase from *Thermomyces lanuginosus*, and even after 48h of reaction there was no production of X1 (xylose). This result is due to the action of purified endoxylanase from *Thermomyces*, which randomly hydrolyzes the β 1,4 bonds of xylan[9]

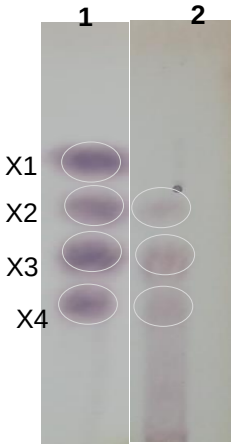

Figure 2 TLC analysis of the hydrolysis products (2) from corn straw hemicellulose produced by purified endoxylanase of *Thermomyces lanuginosus* PC7S1T after 48 h of reaction compared to standard (1) (X1-xylose, X2-xylobiose, X3-xilotriose and X4-xylotetraose).

4 CONCLUSION

Corn straw hemicellulose, an abundant residue from the Cascavel-Paraná region, showed to be an efficient substrate for XOS production using endoxylanase from *Thermomyces lanuginosus* PC7S1T. The analysis of the XOS obtained from corn straw hemicellulose after hydrolysis by endoxylanase was predominantly composed of xylobiose, xylotriose and xylotetraose, with no detection of xylose, indicating targeted breakdown into valuable oligosaccharides. Additionally, these yields can be enhanced by further optimizing the hydrolysis process, thereby increasing its feasibility for the conversion of lignocellulosic residue into valuable prebiotic compounds.

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