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RESEARCH ARTICLE
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Comparison of Four Cereal Straws as a Substrate for Obtaining Xylooligosaccharides using Hydrolytic Enzyme Extracts from *Trichoderma harzianum*

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ABSTRACT

The waste generated from the cereal harvest has great biotechnological potential since it can be used as raw material to produce compounds with greater added value. This work studied four cereal straws (Oats, Wheat, Barley, and Corn) as a source of xylooligosaccharides (XOS) using enzyme extracts produced by *Trichoderma harzianum* in solid-state fermentation. Each straw under study was chemically characterized, observing that the greatest amount of holocellulose was obtained in wheat straw at 71.31%. In comparison, the greatest amount of lignin was observed in oat straw at 17.15%. The straws were subjected to an alkaline pretreatment to reduce the amount of lignin, obtaining the highest delignification in wheat straw with 53.27%. *T. harzianum* produced the highest amount of hydrolytic enzymes when it was grown on wheat straw, while when oat straw was used as a substrate, the generation of this type of enzyme was minimal. It was observed that the greatest diversity of XOS was when the hydrolysis was carried out with wheat straw, observing that the lignin present in the residue is the main obstacle to improving these processes.

Keywords: Cellulase, Cereal straws, *Trichoderma harzianum*, Xylanase, Xylooligosaccharides

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INTRODUCTION

Lignocellulosic biomass is one of the most abundant materials in the world; in fact, it is considered the main renewable resource on the planet; its production is approximately 200,000 million metric tons annually (Ahmad et al., 2019). There is great interest in using this biomass from the point of view of a useful resource that can be converted into energy and food for animals (Park and Sim, 2012). Within lignocellulosic residues, straws are part of the cereal plants that remain after the grain has been harvested and comprises approximately half of the total dry weight of the crop (Chen et al., 2008). In general, for every ton of cereal production, about 1.5 tons of straw are produced as a by-product (Yuan and Sun, 2010). A large part of cereal straws are used for the production of biofuels (Zabed et al., 2016), for the generation of various products such as biogas (Yu et al., 2019), methane (Jackowiak et al., 2011), cellulose nanocrystals (Thakur et al., 2020), syngas production (Okolie et al., 2020) and as a supplement in livestock feed due to its high fiber content (Praveen et al., 2023). The chemical composition of these wastes favors the development of various biotechnological processes, including the obtaining of sugars from the hydrolysis of the cellulose and hemicellulose present (Chen et al., 2023; Khantibongse and Ratanatamskul, 2023). However, the elimination of lignin is the main biotechnological challenge, which is why a great diversity of methods have been developed for its removal, highlighting chemical processes (Serna-Díaz et al., 2016; He et al., 2020; Beroual et al., 2021; Muharja et al., 2021; Susi et al., 2023), biological (Quintanar et al., 2012; Wang et al., 2020; Naik et al., 2021), and combination of processes

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physicochemical-biological (Wang et al., 2019; Ibrahim and Kruse 2020; Maia et al., 2021). Another alternative is using lignocellulosic waste as a substrate for producing biomass and hydrolytic enzymes (Serna-Díaz et al., 2020; García-Esquivel et al., 2023). To produce XOS, different types of cereal straws have been used, as well as physical, chemical, and enzymatic processes (Ávila et al., 2020; Isci et al., 2021; Brenelli et al., 2022; Precup et al., 2022; Chen et al., 2023). In the present work, four different types of cereal straws (oats, barley, corn, and wheat) were compared in terms of their chemical composition and ease of generation of XOS using controlled enzymatic hydrolysis with a crude enzymatic extract of *Trichoderma harzianum*.

MATERIALS & METHODS

Raw Material, Cleaning and Conditioning

This study used oat, barley, corn, and wheat cereal straws obtained from Apan (19°39'03" N, 98°25'02" W), Hidalgo, México. The straws were conditioned before use by washing twice with hot water (60°C) for 30min and dried in an oven at 50°C for 24 h. Subsequently, a grinding process was carried out to reduce the particle size. The samples were sieved with mesh sizes 40 (0.425mm) and 30 (0.300mm). All washed and sieved straws were stored in hermetically sealed plastic bags and kept in a dark room until use.

Chemical Analysis of Cereal Straws

Extractable Compounds in Organic Solvents

The TAPPI 204 om-88 standard was followed. Particle size number 40 (0.425mm) was used. The percentage was determined by weight difference, with a precision of 0.1mg.

Hot Water Removable Compounds

The sample resulting from the extraction with organic solvents was subjected to the TAPPI 207 om-93 standard to calculate the content of compounds extractable in hot water. The percentage of extractable components in water was obtained by comparing the sample's weight before and after extraction.

Ash Content

The TAPPI 211 om-93 standard was used. This analysis used 2g of each straw with a particle size of 40. The percentage of ash was determined by weight difference.

Determination of Acid-insoluble Lignin

The TAPPI 222 om-88 standard was carried out from 1g of sample free of total extractables. The percentage of lignin was determined by the difference in weight between the sample and the final weight of the process.

Determination of Holocellulose

The holocellulose content of cereal straws was determined according to the technique of Browning (1967). Two grams of sample was weighed into a 250mL Erlenmeyer flask, and 63mL of deionized water was added. 0.2mL of glacial acetic acid and 0.6g of sodium chlorite were added to the suspension. Consecutively, it was covered with a watch glass and placed in a water bath (70-80°C) for 1h. This process was repeated two more times. At the end of the third hour, the flasks were placed in an ice bath until the temperature reached 10°C, filtered, and washed with deionized water. The sample was dried at 60°C and weighed. The holocellulose content was determined by weight difference considering the percentage of humidity and organic matter of the final solid.

Determination of α, β and γ-cellulose

The determination was carried out using the TAPPI 203 om-88 standard. A sample of 1.5g of the material free of extractives was used and was subjected to the determination of holocellulose.

Propagation and Conservation of the Strain

The filamentous fungus *T. harzianum* (GenBank access code JQ080073) was obtained from the strain collection of the Aprovechamiento Integral de Recursos Bioticos Laboratory of the Polytechnic University of Pachuca. The strain was grown on Potato and Dextrose Agar (PDA) plates at 28°C for seven days or until sporulation. The strain was kept at 4°C until the subsequent replanting.

Hydrolytic Enzyme Production

Every 12h, three flasks containing the cereal straws inoculated with the conidiospores solution were taken and analyzed by determining the cellulolytic and xylanolytic activities. The following process was followed to obtain the crude enzyme extract. 20mL of distilled water was added to each flask. The flasks were shaken at 150rpm for 30min. Subsequently, the extracts were centrifuged at 10,000 rpm for 10min at 4°C. The enzymatic crude extracts (ECE) thus obtained were stored in sterile 15mL tubes and kept at 4°C until analysis. The cellulolytic activity was determined by the 3-5, dinitrosalicylic acid method modified by Loera and Córdova (2003), which consists of placing 450µL of 0.2% carboxymethylcellulose (CMC) as a substrate, previously dissolved in acetate buffer (100mm, pH 5.3) in a test tube; subsequently 50µL of the crude enzymatic extract of the straw was added and kept incubated at 40°C for 20min. After a time, 750µL of DNS was added, and this mixture was boiled for 10min in a water bath and then cooled in an ice bath. The enzymatic activity was obtained by spectrophotometry at 640 nm. The enzymatic activity was measured by subtracting the absorbance value from the reducing sugars generated. Distilled water was used as the reaction blank, replacing the 0.2% CMC, treated under the same conditions.

The xylanolytic activity was determined by the 3-5, dinitrosalicylic acid method modified by Loera and Córdova (2003), which consisted of placing 450µL of 0.2% birch xylan as a substrate, previously dissolved. In acetate buffer (100mm, pH 5.3) in a test tube, 50µL of crude enzyme extract was added and kept incubated at 40°C for 20min. After, 750µL of DNS was added and brought to a boil for 10min in a water bath, then cooled in an ice bath. The samples were in a spectrophotometer at 640nm. The enzymatic activity was measured by

subtracting the absorbance value from the reducing sugars generated. Distilled water was used as the reaction target, replacing 0.2% birch xylan, treated under the same conditions.

Obtaining Xylooligosaccharides

As the first stage of the process to obtain XOS, the straws were subjected to delignification using an alkaline pretreatment using 2% NaOH in a liquid-to-solid ratio of 6:1 (mL/g of straw) while stirring (200 rpm) for 12h (Ai et al., 2005). Subsequently, the straws were dried for 24h at 60°C and subjected to crushing until obtaining a particle size between 60 (0.25mm) and 80 (0.18mm). The delignified residue was then subjected to a controlled enzymatic hydrolysis process using the crude enzymatic extracts. This controlled process, designed to manipulate the solid: liquid ratio and the ECE concentration, was guided by a 3^2 factorial experimental design. The temperature kept constant (28°C). The hydrolysates were analyzed for reducing sugars using the 3-5 dinitrosalicylic acid method, total sugars (Dubois et al., 1956) and quantification of XOS by HPLC. The lignin content was evaluated using the TAPPI 222 om-88 standard to evaluate the efficiency of delignification, as mentioned before.

High-Performance Liquid Chromatography (HPLC)

The sugars released during the enzymatic hydrolysis process were quantitatively analyzed on an HPLC (Thermo Scientific) coupled to a refractive index (RI) detector. The hydrolysates were filtered using a Millipore cellulose nitrate membrane (0.2μm). A Rezex RSO-Oligosaccharides Ag$^{\pm}$ 4% column (LC 200 X 10mm, Phenomenex) maintained at 80°C was used. HPLC grade water as mobile phase with a flow of 0.3mL/min and 5 μL of sample was injected per analysis. The concentration of each oligosaccharide was quantified using the half-maximum surfaces compared to the peak areas of the standard XOS. Xylose, xylobiose, xylotriose, xylotetrose, and xylopentose (Megazyme, Ireland) were used as standards at a concentration of 10mg/mL, serving as reference points for accurate quantification.

Data analysis

The total results obtained (the three repetitions of each of the treatments) were analyzed using a one-way analysis of variance ANOVA coupled to the post-hoc test (Tukey) with a $p<0.05$, using the Minitab software version 17. The Factor Analysis was carried out with the help of Statistica software version 13.

RESULTS

Chemical Analysis

The results of the chemical analysis are shown in Table 1. The highest amounts of total extracts were obtained from straw of oat (19.97±0.19) and barley (20.45±0.36), while the lowest amount was observed from wheat straw with 10.85±0.09%. The largest amount of lignin was in oat straw, with 17.15±0.17%. Regarding the holocellulose content, the sum of cellulose and hemicellulose, the highest amount was detected in wheat straw with 71.31±0.32%, while the lowest amount was detected in oat straw with 58.44±0.03%. This parameter is relevant because it can be taken as an indication of the potential of the raw material as a source of carbohydrates.

Table 1: Chemical composition of barley, corn, oat, and wheat straws.

Parameter (%)	Substrate			
	Oat	Barley	Corn	Wheat
Moisture	8.90±0.12^{c}	9.70±0.01^{a}	8.60±0.08^{c}	9.10±0.11^{b}
Total extracts	19.97±0.19^{a}	20.45±0.36^{a}	17.43±0.14^{b}	10.85±0.09^{c}
- In organic solvents	4.45±0.06^{a}	3.19±0.69^{b}	3.72±0.17^{ab}	3.63±0.11^{ab}
- In hot water	15.52±0.33^{b}	17.26±0.02^{a}	13.71±0.10^{c}	7.22±0.11^{d}
Ash	6.30±0.11^{b}	5.28±0.15^{c}	7.53±0.07^{a}	3.79±0.14^{d}
Lignin	17.15±0.17^{a}	10.89±0.05^{d}	14.00±0.14^{c}	15.83±0.09^{b}
Holocellulose	58.44±0.03^{c}	65.21±0.03^{b}	61.22±0.15^{c}	71.31±0.32^{a}

The different letters (in rows) indicate significant statistical differences according to the ANOVA and test Tukey ($p<0.05$). ± = Standard deviation. The experiments were performed in triplicate.

Holocellulose is a term that refers to the total carbohydrate content (cellulose and hemicellulose), composed of α-cellulose (crystalline cellulose), β-cellulose (amorphous cellulose) and γ-cellulose, which mainly represents hemicelluloses. Table 2 shows the results of the holocellulose analysis. β-cellulose was observed in all wastes, with the highest concentration in wheat straw at 38.99±0.16%. The most hemicellulose (γ-cellulose) was also observed in wheat straw with 28.84±0.15%. All straws showed significant differences regarding holocellulose analysis.

Table 2: Analysis of the holocellulose composition of the four straws used.

Parameter (%)	Substrate			
	Oat	Barley	Corn	Wheat
Holocellulose	58.44±0.03^{c}	65.21±0.03^{b}	61.22±0.15^{c}	71.35±0.32^{a}
a) α-cellulose	31.92±0.07^{b}	30.50±0.07^{c}	30.82±0.13^{c}	38.99±0.16^{a}
b) β-cellulose	2.67±0.08^{c}	16.02±0.02^{a}	8.76±0.09^{b}	3.52±0.11^{c}
c) γ-cellulose	23.64 ±0.10^{b}	18.69±0.04^{c}	21.64±0.05^{b}	28.84±0.15^{a}

The different letters (in rows) indicate a significant statistical difference according to the ANOVA and Tukey test ($p<0.05$). ± = Standard deviation, experiments were performed in triplicate.

Kinetics of the Production of Hydrolytic Enzymes (cellulases and xylanases)

Cellulolytic and xylanolytic activity kinetics were carried out using the four straws under study as substrate and *T. harzianum*. The results are presented in Fig. 1A. It was observed that the best substrate to produce xylanases was wheat straw since after 84h of growth of *T. harzianum* on this substrate, 44.14 AU (Activity Unit)/gdm (gram of dry matter) was detected, while the highest cellulolytic activity was observed when it was used as substrate barley straw with 8.23AU/gdm. In all treatments, the xylanolytic activity was observed to be greater than the cellulolytic activity. On the other hand, when taking the concentration of extracellular protein (Fig. 1B) as an indirect growth parameter, no significant difference was observed in the fungus growth using barley and corn straw as a substrate. The lowest production of extracellular protein was observed in oat straw, which also coincides with the lowest concentration of holocellulose detected. In contrast, the highest extracellular protein production was detected when the fungus was grown on wheat straw.

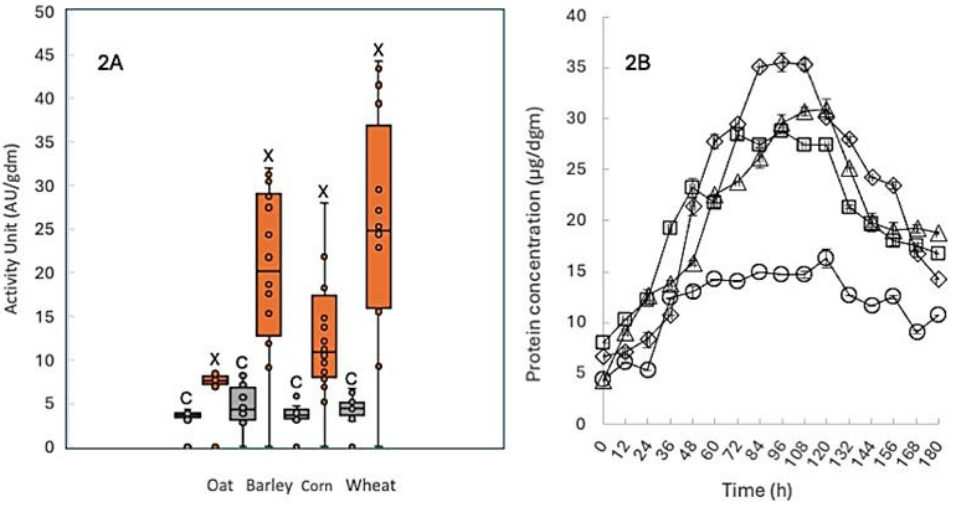

Fig. 1: Production of hydrolytic enzymes (A) and extracellular protein (B) in solid fermentation using the four cereal straws and *Trichoderma harzianum*. C = Cellulases; X = Xylanases. ▪ = Barley, ▪ = Corn, ▪ = Oat, ◊ = Wheat.

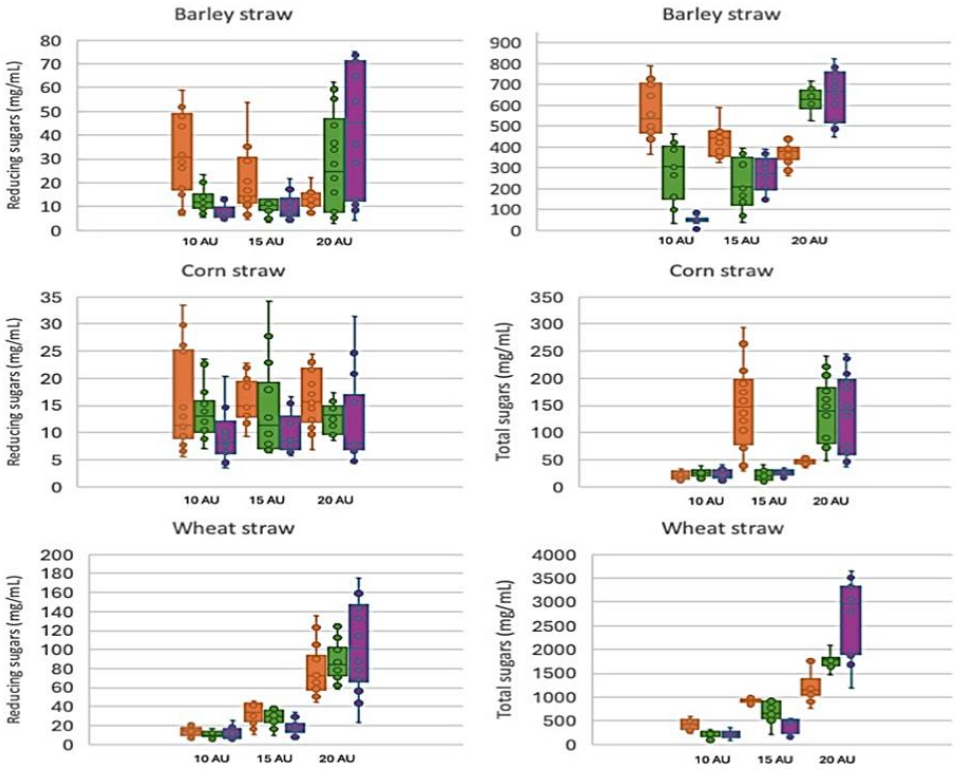

Fig. 2: Reducing and total sugars released during the enzymatic hydrolysis of the three selected cereal straws using three concentrations of enzymatic activity units (10, 15, and 20) of the enzymatic crude extracts produced by *Trichoderma harzianum*. ▪ = Dil 1:20, ▪ = Dil 1:50, ▪ = Dil 1:100.

Pretreatment of Cereal Straws

As a previous step to the enzymatic hydrolysis, an alkaline pretreatment was carried out to solubilize the greatest amount of lignin in these wastes. Table 3 shows the results obtained. The maximum lignin removal was 53.27% in wheat straw, followed by oat straw with 51.96% removal. Finally, the removal values for corn and barley straw were 46.49 and 37.49%, respectively.

Table 3: Values obtained from the chemical delignification of the studied cereal straws.

Parameter (%)	Substrate			
	Oat	Bayley	Corn	Wheat
Initial lignin	17.15±0.17	10.89±0.15	14±0.24	15.83±0.39
Final lignin	8.91±0.29	4.08±0.36	6.51±0.23	8.43±0.41
Removal	51.96	37.49	46.49	53.27

± Standard deviation

Enzymatic Hydrolysis of the Cereal Straws

For this stage, oat straw was eliminated as it was the substrate where the lowest concentration of holocellulose and hydrolytic activity was detected. The results obtained from the hydrolysis using the crude enzyme extracts are shown in Fig. 2. The greatest release of reducing sugars was observed in treatment with wheat straw at the 1:100 dilution with 20AU/mL, with a maximum value of 164mg/mL of reducing sugars. The second-best condition was observed in barley straw at the 1:100 dilution and 20AU/mL, where 75.1mg/mL of reducing sugars were obtained. Otherwise, with corn straw, the best condition for releasing reducing sugars was with the 1:20 dilution and 10AU/mL; no differences were observed in the different treatments with corn straw regardless of the conditions used. In the treatments using barley, corn, and wheat straw, a trend in the hydrolysis of hemicellulose and cellulose was observed when 10 and 15AU/mL of enzyme extract were used, probably due to an inhibition effect due to the substrate. In the treatments where 20AU/mL were used, this trend was reversed, with the treatments with the highest sugar release being those with the highest dilution

and concentration of enzyme extract. Regarding total sugars, the behaviors were very similar with the greater presence of sugars. Again, in the treatment of wheat straw, and 20AU/mL and dilution 1:100, the highest concentration of total sugars was detected with 3657.5mg/mL, while the lowest release conditions of total sugars were for the straw treatments of corn.

Xylooligosaccharides Production

The best hydrolysis condition identified in the previous section was chosen to carry out the controlled enzymatic hydrolysis of barley, corn, and wheat straws. Hydrolysis kinetics were carried out, and the hydrolysates were analyzed using HPLC to identify the oligosaccharides released during each treatment. Table 4 shows the best results obtained from the kinetics of the enzymatic hydrolysis of the straws of the three cereals. The greatest diversity of XOS was observed in the treatment of barley straw in the condition 20AU/gdm, dilution 1:20, for 40 h; in this condition, xylose (X), xylobiose (X2), xylotriose (X3), xylotetrose (X4) and xylopentose (X5) were detected with 70.8, 36.4, 16, 14.6 and 6.2mg/gdm, respectively. In the case of corn straw, the diversity of XOS was lower since, in the two treatments carried out, only the presence of X, X2, and X3 was observed. In the case of wheat straw, it was the most susceptible to hydrolyzing to monosaccharides since it was where the greatest presence of xylose was detected.

Table 4: Xylooligosaccharides produced by the hydrolysis of three cereal straws and ECE of *T. harzianum* identified by HPLC

Straw	Condition	Xylooligosaccharide type	Concentration (mg/gdm)
Barley	20AU/gdm	X	32±0.06
	1:100	X2	19±0.01
	20 h	X3	15±0.01
		X4	41±0.06
	20AU/gdm	X	39±0.04
	1:100	X2	36±0.00
	32 h	X3	15±0.01
		X4	13±0.01
	20AU/gdm	X	53±0.04
	1:100	X2	66±0.08
	40 h	X3	13±0.02
		X4	45±0.01
	20AU/gdm	X	70.8±0.06
	1:20	X2	36.4±0.02
	40 h	X3	16±0.03
		X4	14.6±0.04
		X5	6.2±0.03
Corn	15AU/gdm	X	39±0.03
	1:50	X2	5.5±0.01
	20 h	X3	7.5±0.02
	10AU/gdm	X	6.5±0.02
	1:50	X2	18.5±0.01
	60 h	X3	7.5±0.01
Wheat	20AU/gdm	X	773.9±0.35
	1:100	X3	97±0.05
	24 h		
	20AU/gdm	X	831.3±0.41
	1:100	X2	21±0.01
	32 h	X3	62±0.04
		X4	75±0.03
	20AU/gdm	X	111.89±0.13
	1:100	X3	25±0.03
	40 h		
	20AU/gdm	X	133.98±0.11
	1:100		
	48 h		

Table 5: Generation of xylooligosaccharides from the hydrolysis of wheat straw using enzymatic extracts of *T. harzianum* with different hydrolysis times

Condition	Type of xylooligosaccharides	Concentration (mg/gdm)
20AU/gdm, 1:20, 24 h	X	98.92±0.23
	X3	6.2±0.04
20AU/gdm, 1:20, 32 h	X	105.19±0.71
	X2	5.4±0.01
	X3	7.8±0.13
20AU/gdm, 1:20, 40 h	X	108.51±0.11
	X2	3.8±0.02
	X3	7±0.08
	X4	1.8±0.01
20AU/gdm, 1:20, 48 h	X	122.85±0.63
	X2	4.4±0.19
	X3	8.8±0.02
	X4	2.4±0.01
20AU/gdm, 1:20, 60 h	X	111.34±0.26
	X2	3.2±0.01
	X3	5.6±0.04
	X4	23±0.15

Wheat straw was the most susceptible to enzymatic hydrolysis, so it was again subjected to enzymatic hydrolysis, decreasing the hydrolysis time to determine if the dilution affected hydrolysis and to have greater control over it. Five treatments were carried out: T1 (20AU/dgm, 1:20, 24 h), T2 (20AU/dgm, 1:20, 32 h), T3 (20AU/dgm, 1:20, 40 h), T4 (20AU/dgm, 1:20, 48 h) and T5 (20AU/dgm, 1:20, 60 h), the results are shown in Table 5. In this case, the presence of X2 and X3 was observed in all treatments longer than 32h (Treatments 3, 4, and 5) (Fig. 3A). The production of X4 in treatment five was 1.15mg/mL, followed by the production of X3 and X2. It was observed that the longer the hydrolysis time, the greater the amount of xylose released (Fig. 3B). The results showed 98.92, 105.2, 108.5, 122.85, and 111.34mg/dgm of xylose released in treatments T1, T2, T3, T4 and T5, respectively.

DISCUSSION

Significant differences were observed in the chemical composition between the different straws studied. Similar results have been reported when the chemical composition of straws of different origins is studied; thus, Ai et al. (2005) carried out a study with corn straw and reported a moisture content of 8.9%, while Gupta et al. (2012), reported a moisture content of 8% for corn cobs. Regarding the content of total extracts, significant differences were observed concerning compounds soluble in solvents (non-polar compounds) and water (polar compounds). The water-extractable compounds are mainly formed by non-structural compounds such as tannin residues, amino acids, carbohydrates, and alcohols (García-Esquivel, 2023), while the solvent-extractable compounds are mostly made up of waxes, fats, tannins, resins, sterols, and proteins (Sumathi and Hung 2004). For biological hydrolysis processes using enzymes, eliminating compounds such as tannins, alcohols, and resins that can act as inhibitors of this type of enzyme is very important (Zhai et al., 2022). The highest extraction of non-polar compounds was observed in oat straw, and the highest extraction of water-soluble compounds was detected in barley straw. Microorganisms can initially use non-structural compounds to initiate the growth process on

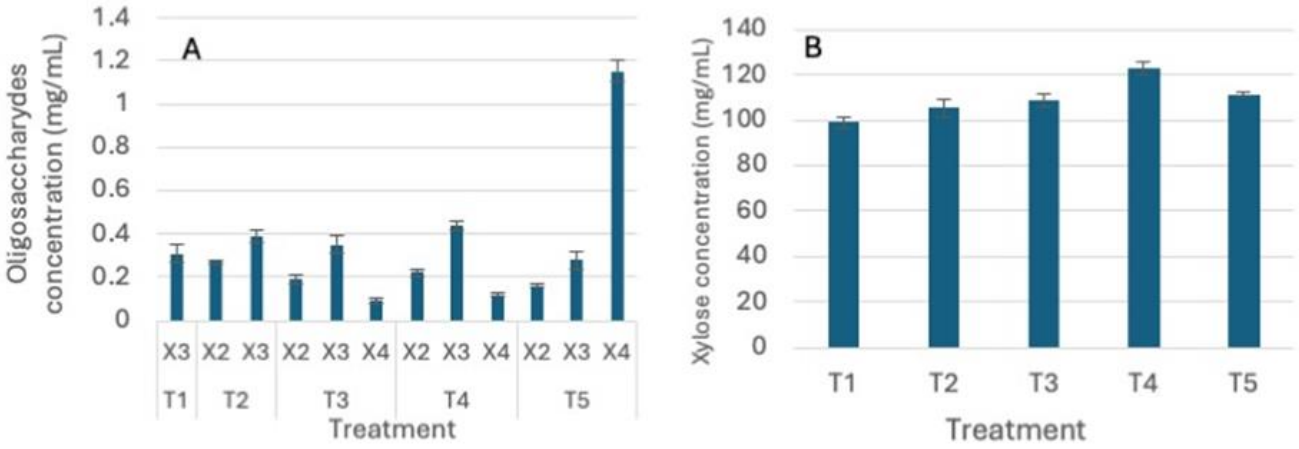

Fig. 3: Production of xylooligosaccharides in the different enzymatic hydrolysis treatments (A). Production of xylose as a product of enzymatic hydrolysis in the different treatments (B).

lignocellulosic waste since they can be used as an easily assimilated carbon source. The results obtained are similar to those reported in other works; Barroso (2010) reported 18.65% of wheat straw; of total extract compared to the present study, 10.85% were obtained.

Regarding the ash content, Pronyk and Mazza (2012) mention in their study the ash content for lignocellulosic waste that ranges around 1-20%, of which 65-70% is silica, which represents an impurity and mineral material that usually causes problems in the processing of raw materials. Similar values have been reported for wheat straw ash with 4% (Pierre et al., 2011), rice straw, and corn cob with 16.34 and 1.32%, respectively (El-Zawawy et al., 2011). All values obtained from the study are within the range mentioned above. Regarding the ash content, silica can favor its increase, as happens in cereal straw waste, a situation that does not occur in other types of lignocellulosic waste.

Holocellulose represents the total fraction of polymeric carbohydrates, the sum of cellulose plus hemicellulose integrated into the cell wall together with lignin. The holocellulose content indicates the potential of the waste under study since microorganisms can potentially use carbohydrates as a carbon source during their growth on the lignocellulosic waste (Jatoi et al., 2021). The holocellulose content is usually greater than 50% of the dry weight of the lignocellulosic waste. Adapa et al. (2009) obtained 53.61% holocellulose for barley straw, the value of this research being higher than that mentioned by the authors. However, holocellulose values of 71.5% for oat straw and 70% for barley straw have been reported (Román-Gutiérrez et al., 2022). It is known that factors such as climate, species, cultivation practices, crop age, and soil type, among others, have a strong impact on the composition of lignocellulosic waste (Liu et al., 2010; Yuan and Sun, 2010), which can affect the chemical composition of the same species.

Regarding lignin content, oat straw has the highest content of this biopolymer, 17.15%, followed by wheat, corn, and barley straw. Yuan and Sun (2010) report a lignin content for oat straw of 17.5%, with the lignin range for lignocellulosic waste being between 15-20%. On the other hand, Chandel and Singh (2011) found 23.4% lignin in wheat straw, while Adapa et al. (2009) reported 12.85% for oat straw. The presence of lignin affects enzymatic hydrolysis since it forms a very chemically complex matrix with holocellulose, preventing the microorganism from having access to the carbohydrates of the substrate (Hendriks and Zeeman, 2009). The ether and alkyl bonds of lignin are not susceptible to hydrolytic attacks; therefore, it is very resistant to biological and chemical degradation (Sifontes and Domine, 2013). Therefore, to improve carbohydrate yields it is necessary to apply chemical pretreatments to eliminate the greatest amount of lignin and improve enzymatic and chemical hydrolysis.

Various pretreatment methods have been applied to cereal straws to improve carbohydrate release. Xu et al. (2007) reported the use of ammonia as a pretreatment of soybean straw followed by enzymatic hydrolysis, observing a decrease in lignin concentration of 30.16%. Asghar et al. (2015) mentioned 83% delignification of wheat straw using an alkaline process. On the other hand, Ben'ko et al. (2020) reported 90% delignification of wheat straw using ozonation. While Ibarra-Díaz et al. (2022) reported 60% delignification of barley straw using an alkaline peroxide method, and Sun et al. (2002) reported up to 72.3% delignification of barley straw using a similar method. Regarding oat straw, Agu et al. (2017) reported 48% delignification using microwave-assisted alkaline pretreatment. Karki et al. (2011) used aqueous ammonium to delignify oat straw and observed up to 48.1% delignification. Delignification methods have also been explored for corn straw. Gunam et al. (2020) reported 61.45% delignification for corn straw using an alkaline treatment with NaOH. Quintanar et al. (2012) also using an alkaline treatment, obtained 34% delignification for corn straw. These results contrast with what was described by Serafín-Muñoz et al. (2019), who obtained a delignification of corn straw of 93.4% using a combined NaOH and H_2O_2 method. The results obtained in the present work regarding delignification are like the previously mentioned values since they ranged from 37.49 to 53.27% delignification using only NaOH (2%) and room temperature, representing energy advantages. It is evident that the extraction conditions have a strong impact on the lignin solubilization processes, where alkaline methods stand out, whose function is to partially depolymerize the

lignin, as well as partly facilitate the separation of hemicelluloses from cellulose, making it more available. for subsequent processes (Ruiz et al., 2023).

T. harzianum has been used to generate hydrolytic enzymes by applying them to the hydrolysis of wheat straws (Carvalho et al., 2013; Chaudhary et al., 2021), barley (Dekker 1989) and maize (Yegin 2023; Ghorai 2024) to produce XOS and has not been used for the hydrolysis of oat straw. Obtaining oligosaccharides using cereal straws is favored by the high xylan content, which makes this type of lignocellulosic waste an excellent raw material for generating molecules with greater added value.

Hydrolyzed hemicelluloses can be a source of fermentable carbohydrates or for the generation of furan-based compounds (Aristizábal et al., 2015; Michelin and Teixeira, 2016). Sun and Tomkinson (2000) identified a great diversity of carbohydrates present in various lignocellulolytic residues, including barley oats, wheat, and corn straws. They observed that the carbohydrate found in the greatest quantity is xylose, which is the monomer of xylan, which makes this type of waste an excellent candidate for obtaining XOS. Currently, partially hydrolyzed hemicelluloses are an excellent source of XOS that have applications in the health field as prebiotics (Álvarez et al., 2022). Chemical, physical, and biological methods have been used to produce XOS using cereal straws. Álvarez et al. (2022) applied steam explosion to barley straw, obtaining yields of 23.1g/L of XOS and other sugars such as glucose, arabinose, mannose, and galactose. For their part, Huang et al. (2017), using hydrolytic enzymes and previously pretreated wheat straw, obtained xylobiose and xylotriose with yields of 53 and 20mg/g, respectively, as byproducts of the alcoholic fermentation of wheat straw hydrolysates.

Azzouz et al. (2021) used the enzyme extracts produced by *Aspergillus niger* during its growth on wheat straw to produce XOS, obtaining xylose, xylobiose, xylotriose, xylotetrose, and xylopentose in addition to other monosaccharides, with time being the main factor to control. Akpinar et al. (2009) from wheat straw pretreated with alkali and a controlled acid method generated XOS. The results indicated that at low concentrations of H_2SO_4 a mixture of xylan oligosaccharides of 1 to 6 carbon atoms is obtained in a time of 30min in addition to other monosaccharides. An advantage of using acids is that the hydrolysis time is generally shorter than when hydrolytic enzymes are used, which is probably an advantage; however, there is no precise control of hydrolysis, which can generate excess hydrolysis of the acids of xylose polymers, even producing enzyme-inhibitory compounds such as furfurals (Akpinar et al., 2009).

On the other hand, Precup et al. (2022) used a combined barley straw pretreatment process that included a hydrothermal pretreatment followed by an alkaline pretreatment to finally use enzymes produced by *Trichoderma viride*, obtaining a complex mixture of XOS, mainly xylotriose and xylotetrose and other monosaccharides many of them, considered as non-digestible fiber (Precup et al., 2022), therefore, it is of utmost importance to control the hydrolysis rate to favor the presence of XOS.

The results obtained in the present work indicate the potential that *T. harzianum* has to be used for the production of XOS since it has the enzymatic battery to carry out the hydrolysis of xylan present in cereal straw residues (Cacais et al., 2001; Azimova et al., 2020) and can also be enhanced if the enzyme extracts are obtained from the growth of this fungus on the waste (Namasivayam et al., 2015; Marques et al., 2018) since they can function as inducers of hydrolytic activity. Of the four residues studied, *T. harzianum* did not show outstanding hydrolytic activity in oat straw, which also coincided with the lowest levels of holocellulose detected of the four residues and with the highest amount of lignin; these factors influenced the growth of the fungus and therefore, in lower production of enzymes and hydrolysis of hemicelluloses. It has been reported that lignin has a protective effect on hemicellulose that prevents hydrolytic enzymes from having free access to this biopolymer (Goyal et al., 2008). This statement will be reinforced with the results of the other cereal straws since the greatest diversity of XOS was observed in barley straw, which, after chemical delignification, detected 4% of lignin and that, the smallest amount of lignin, it allows greater access to xylan and therefore, greater hydrolysis of it.

Conclusion

Chemical differences were observed in the four cereal straws studied, which impacted the enzymatic hydrolysis processes. Oat straw presented the highest percentage of lignin, which influenced a lower growth of *T. harzianum* and hydrolytic activity. The greatest diversity of XOS was obtained from barley straw, which coincided with the lowest lignin content. This indicates that for the hydrolysis processes of hemicelluloses, as is the case of xylan, the lignin present in the lignocellulosic residue plays a crucial role in improving production processes. Therefore, to produce XOS, it is essential to optimize delignification processes to obtain the best yields of this type of sugars.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Authors' Contributions: CSYE Performed the study. TJA and RGAD Conceived and designed the experiment. ARMA and TJX Conducted lab analyses. MFY and TJX Performed statistical analyses. CSYE, RGAD and TJA Prepared the draft of the manuscript.

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