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ORIGINAL RESEARCH ARTICLE

Valorization of (*Hyphaene thebaica* L) 'Goruba' Fruit Waste for Optimized Prebiotic Xylooligosaccharides Production via *Aspergillus flavus* Xylanase

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ABSTRACT
Xylooligosaccharides (XOS) are a prebiotic fiber obtained from the hydrolysis of xylan, found in plant cell wall. Benefits of consuming it include regulating blood sugar, managing high blood pressure, and reducing bad cholesterol, which is responsible for about 30% of deaths in developing countries. XOS was produced and optimized from 'Goruba' fruit waste using the response surface method aimed at adding value to the residue. The alkaline method was used to extract xylan from the sample, and the medium containing the xylan was used to produce xylanase from *Aspergillus flavus* under solid-state fermentation (SSF). Xylanase was assayed using the DNS method, and the hydrolyzed xylan to XOS was analyzed using HPLC. One-way ANOVA ($p < 0.05$), mean ± SD, and numerical optimization were used to analyze the data. The highest xylan yield was 34.35±1.02% with 9.0% NaOH. Crude xylanase after SSF was 362.3 mg/mL, total activity (2829 IU), and specific activity (7.81 IU/mg). Ammonium sulphate precipitated protein (41.17 mg), total activity (2737 IU), specific activity (66.48 IU/mg), and yield (96.74%). Xylanase exhibited optimal activity of 22.33 at 50 °C and pH 6.0 after 4 hours. The HPLC fractions generated xylose (0.946), xylobiose (0.603), xylose (0.323), xylotetrose (0.356), and xylopentose (0.184) with 24.93 mg/mL of XOS. Numerical optimized XOS was 26.05 mg/mL at 52 °C, pH (7.90) at 2 hours, enzyme dose (2 mg/mL), and substrate dose (8.50 mg/mL). The amount of XOS produced has established that the biomass is a good source for XOS (prebiotics). Conclusively, biomass can be an alternative for the large-scale production of XOS at a low cost due to its affordability and availability.

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INTRODUCTION
Xylooligosaccharides (XOs) is a rapidly growing class of oligosaccharides with a broad range of applications as prebiotics, used in cosmetics, agriculture, medicines, and in the food industry (Howard *et al.,* 2020). XOs are classified as complex sugars that the body cannot digest; they are mainly derived from the hydrolysis of xylan, a component of lignocellulosic materials found in plant cell walls. The XOs contain xylose as monomers with a unit chain of 2 to 10 (Chen *et al.,* 2020). As a prebiotic and functional food, it offers numerous health and nutritional benefits, including lowering blood pressure and cholesterol, promoting gastrointestinal well-being, exhibiting anti-cancer properties, and regulating blood sugar (Parakh *et al.*, 2020). Advancements in the healthcare sector have led to an increasing recognition of xylooligosaccharides as a superior alternative to other functional foods and prebiotics due to their low additive content, superior stability, and high selectivity. They have been proven to be bacteriostatic and useful in improving the productivity of some domestic animals. (Sonal, *et al.*, 2019 and Azzouz, *et al.,* 2021). Deficiencies associated with a lack of taking prebiotic fiber include digestive disorders, weakness of the immune system, an increase in high blood pressure, and type II diabetics, which may result to cardiovascular diseases (Khat-udomkiri *et al.,* 2020)..Challenges facing the production and application of XOs include addressing the problems of production, lack of awareness for local consumption, marketability, and optimization of raw materials and production conditions using local raw materials (Howard *et al.,* 2020). Despite extensive studies on lignocellulosic agro-industrial residues such as wheat straw, corn cob, and sugarcane bagasse for xylooligosaccharide (XOS) production using microbial xylanases, the valorization of *Hyphaene thebaica* ("Goruba") fruit waste remains virtually unexplored. Goruba, widely available across sub-Saharan Africa, has

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been shown to contain substantial xylan but has not been systematically assessed as a feedstock for tailored A. *flavus* xylanase hydrolysis. Moreover, existing optimization efforts focus primarily on generic substrates, leaving a lack of substrate-specific design of experiments, enzyme kinetics characterization, and functional evaluation of the resulting XOS. Addressing these gaps is essential to establish Goruba as a sustainable, high-value source of prebiotics and to define operating parameters that maximize yield, purity, and bioactivity

MATERIALS AND METHOD

Materials and Sample Preparation

Xylose, xylobiose, and other standard sugars were purchased at SRL (Sisco Research Laboratories) Pvt. Ltd. 608-B, (East), Mumbai India. Other materials and consumable reagents were obtained locally. A pure culture of *Aspergillus flavus* was obtained at the Department of Microbiology, Modibbo Adama University, Yola. Adamawa State. Pulp of ripened and dried 'Goruba' fruit was removed, washed and boiled with portable water to obtain the chaff. The chaff was drained and oven-dried at 55°C to a constant weight before being milled into flour using roller mills. The flour was sieved through a 0.02 mm sieve and stored in a plastic container in a cool, dry place (AOAC, 2006).

Determination of Hemicellulose

Two (2) g of the 'Goruna' fruit waste flour sample was weighed into a beaker containing 100 mL of neutral-detergent fiber (NDF) reagent containing 30 g/l of sodium lauryl sulphate, 18.61 g/l of disodium ethylenediaminetetraacetatic acid (EDTA) dihydrate, 6.81 g/l of sodium borate decahydrate, 4.56 g/l of anhydrous disodium hydrogen phosphate, 10 ml/l of 2-ethoxyethanol, 2 ml of decahydrophthalene and 0.5 g of sodium sulphite. The mixture was boiled for 60 min before being filtered onto a tared Gooch crucible. The residue was thoroughly washed with acetone and then with boiling water before being dried at 100 °C and weighed to determine the NDF (AOAC, 2006).

$$\text{Hemicellulose content} = \frac{\text{Neutral Detergent Fiber (NDF)}}{\text{Weight of Sample}} \times 100\%$$

Quantification of Xylan

Two (2.0) g of hemicellulose was soaked in a ratio of 1:10 with 3,6,9, 12, and 15% sodium hydroxide solution and kept overnight with constant agitation at 60 °C before it was steamed at 100°C for 3 hours. The steamed sample was centrifuged at 4,000 rpm for 30 minutes. Potassium chloride solution was added to the supernatant to precipitate the xylan. The precipitated xylan was centrifuged, filtered, and oven-dried to a constant weight at 60 °C (Rodrigues *et al.,* 2022).

$$\text{Xylan yield (\%)} = \frac{Dry\ weight\ of\ extracted\ xylan}{Weight\ of\ hemicellulose} \times 100$$

Production of Xylanase from *Aspergillus flavus*

A defined medium containing 1.0% 'Goruba' xylan, 19g agar, 0.05g $MgSO4{\cdot}7H_2O$, 0.005g $CaCl_2$, 0.005g $NaNO_3$, 0.009g $FeSO_4{\cdot}7H_2O$, 0.002g $ZnSO_4$, 0.012g $MnSO_4$, 0.23g KCl, 0.23g KH_2PO_4 and 2g peptone per liter at pH of 5.3 was used for xylanase production under solid state fermentation (Asad., *et al*, 2013) The Media was introduced in 250 ml conical flask and autoclaved for 20 min at 121°C (15 lbs) and cooled before it was aseptically inoculated with Spores from seven-days *Aspergillus flavus*. Fermentation was carried out for seven days at 28 ± 2°C. The crude enzyme was harvested by adding 200 mL of distilled water with a minimum amount of sodium citrate buffer (50 mM, pH 5.3) and homogenized thoroughly. The slurry was filtered through cheesecloth and centrifuged at 4000 rpm for 20 minutes at 4°C. The supernatant was again filtered through Whatman No. 1 filter paper to obtain the crude enzyme (Asad *et al*., 2013).

Estimation of Xylanase Total Protein.

One hundred (100) mg of Coomassie Brilliant Blue G-250 was dissolved in 50 mL of 95% ethanol, and 100 mL of 85% (w/v) phosphoric acid was added. The solution was then diluted to 1.0 L. The dye was filtered through Whatman 1 paper after dissolution. Ten (10) mL crude enzyme samples were pipetted into 1.5 mL polyethene microfuge tubes and diluted to 1, 1:10, 1:100, 1:1000. The volume of γ-globulin standard protein for the calibration curve was made of 10, 20, 30, 40, 5, 60, 80, 100. A blank containing 100 µl of distilled water was pipetted into test tubes. To each of the dilutions apart from the blank, 1.0 mL of the standard protein was added and mixed gently. The absorbance of each sample and the standard protein was measured at 595 nm over the time intervals of 10, 20 30, 40, 50, and 60 minutes (Mughal *et al.,* 2020).

Three-Step Purification of Crude Xylanase

Ammonium sulphate precipitation

Protein precipitation by salting-out technique using ammonium sulphate ($NH_4(SO_4)_2$) solution was carried out with gentle and continuous stirring. This was left overnight, and the precipitate was collected by centrifugation at 10000 rpm for 20 min at 4°C. The precipitate was then dissolved in 50 mM Tris buffer at a pH of 8.60 before dialyzing against the same buffer for 24 hours using cellulose tubing (molecular weight cut-off 13,000 kDa, Himedia LA393-10MT). (Zolfaghar *et al.,* 2019).

Ion Exchange Chromatography.

DEAE-Sephadex A-50 ion exchange chromatography was used to further purify the dialyzed enzyme. Two (*2*ml) of dialyzed xylanase was loaded onto an anion exchange DEAE Cellulose (Sigma-Aldrich Co., USA) column (height of 20cm and with 2.5cm diameter) packed with activated DEAE-cellulose and equilibrated with 50mM Tris buffer (pH 8.3). The dialyzed xylanase sample was eluted with 0.5M NaCl buffer with the flow rate of

1mL/min carried out at 4°C and stored at 4 °C (Zolfaghar *et al.*, 2019)

Affinity chromatography

The purified enzyme obtained from ion exchange chromatography was further purified using Agarose Blue resin chromatography, packed into a 1.5 cm x 10.0 cm column, and equilibrated with 50 mM citrate buffer (pH 5.3). The xylanase sample was introduced in to the column, washed with the same buffer to remove the unbound protein, and then eluted with 1.0 KCl (Fagbohunka *et al.,* 2017)

Xylanase Assay

The xylanase activity was quantified based on the reducing sugars generated by a 3,5-dinitrosalicylic acid (DNS) solution. Different concentrations of standard sugar (xylose) and hydrolyzed xylan samples were prepared at 3, 6, 9, and 12%. All the samples were incubated at 2, 4, 6, 8, and 10 hours before being heated with 3,5-dinitrosalicylic acid to form a red-brown solution. The optical density (OD) of the coloured complex was determined at 540 nm in triplicate. The graph of concentration versus optical density was plotted using MS Excel, and the amount of xylose was deduced from the extrapolated value on the x-axis, using the dilution factor. One unit of xylanase activity was defined as the amount of enzyme that liberates 1 micromole of reducing sugar equivalent to xylose per minute under the assay condition (Rodrigues *et al*, 2022)

Effect of Temperature and pH on Xylanase Activity

The optimum temperature for the conversion of xylan to XOS by Aspergillus flavus xylanase was determined by varying the incubation and assay reaction temperatures from 30, 40, 50, 60, 70, 80, and 90 °C. The effect of pH on xylanase activity was determined by incubating the sample xylan with the xylanase at pH levels of 3, 4, 5, 6, 7, 8, and 9. The ideal pH for the enzyme was determined by altering the buffering conditions with Tris buffer across the pH range (Alokika and Singh, 2019).

Hydrolysis of Xylan to Xylooligosaccharides

Xylooligosaccharides were hydrolyzed from 'Goruba' xylan pellets using *Aspergillus flavus* xylanase in sodium citrate enzyme buffer solution (Akpinar et al., 2019). The process involved using the pH of 3,4,5,6,7,8, and 9 and temperature of 30,40,50,60,70,80, and 90°C, and varying the enzyme dose of 2U, 4U, 6U, 8U, and 10U, where 1U equals 2.65). The samples were incubated in flasks at 150 rpm in a shaker incubator at specific time intervals of 2, 4, 6, and 8 hours. Aliquots were then taken and analyzed (Aachary and Prapulla, 2019). The prebiotic potency of XOS was assessed by enumerating the growth of *Lactobacillus acidophilus* on the original MRS agar and the one modified with XOS obtained from the sample. The modified media contained basic sources of elements such as nitrogen, potassium, sulphur and sources of vitamins (Aachary and Prapulla, 2019).

Characterization of Xylooligosaccharides (XOS)

High-Performance Liquid Chromatography **(**HPLC) (Agilent, USA) was used to determine the concentration of XOS and reducing sugar from the hydrolyzed xylan. The HPLC was equipped with a refractive index detector (PerkinElmer Series 200), set at 0.1 g/100 mL for a 5 mL sample. The system was also equipped with a Shodex Sugar KS-802 packed column (8 mm ID × 300 mm, F6378020) and a RID-10A (Wu et al.), maintained at 65 °C. Samples were eluted with deionized water at a flow rate of 0.5 mL/min and a limit of quantification (LOQ) of 0.7 mg/mL (AOAC, 2006). The sample was filtered using a 0.2 µm membrane (Minigen USA, MG-25020PVDF) before 20 µL was injected with a manual injector. The quantification of XOS was based on a comparison of average peak areas and was compared with the standards XOS (xylose, xylobiose, xylotriose, xylotetrose, and xylopentose). The concentration was expressed in ng/mL and converted to mg/mL (Akpinar *et al.*, 2019). Xylose, glucose, arabinose, and galactose were eluted with 5mM H_2SO_4 as the mobile phase from anion-mediated stationary phase (Bio-Rad Aminex HPX 87H). The Aminex HPX 87H column (300 mm × 7.8 mm) was used at 45℃ with a flow rate of 0.6 mL/min. Analysis was completed in 45 minutes, and the concentration of the sample sugars was determined using average peak areas, which were compared with those of the standard sugars, expressed in mg/mL (Khaire *et al.,* 2021).

Data Analysis and optimization of XOS production

All data used for this study were replicated. Standard deviation, Fit statistics, and one-way ANOVA were used to analyze the data using Design Expert version 13. Five independent variables and one response (dependent variable) were studied using a Response Surface and Box-Behnken design. The five independent variables were temperature (30, 40, 50, 60, 70, 80, and 90 °C), pH (3, 4, 5, 6, 7, 8, and 9), time (2, 3, 4, 5, 6, 7, and 8 hours), and enzyme, and substrateconcentrationsn ranged from2 tod 10 mg/mL. ANOVA was used to evaluate the significance of the experimental data, as indicated by the value, P-value, and lack of fit (Dhaver *et al.*, 2023). The precision of the experimental data was evaluated using fit statistics, the regression model (R^2), and the adjusted regression square (Adj-R^2). The interaction effect of variables on the response was studied using three-dimensional figures. (Fermoso. *et al.*, 2019). The optimal conditions for the yield of XOS were determined using numerical optimization (Kefas *et al.*, 2018)

RESULTS

Lignocellulosic Composition of the Sample

Table 1 shows the lignocellulosic Composition of the 'Goruba' fruit waste sample, which indicates that the sample contains an appreciable amount of hemicellulose. High hemicellulose content in any sample determines the quantity of xylan and hence the Xylooligosaccharides (Saha *et al.*, 2022). Many researchers have published articles on the lignocellulosic components of a wide range

of agricultural leftovers and reported lower amounts of hemicellulose than those obtained in this study.

Quantification of Xylan

Table 2 shows the result of xylan obtained from the 'Goruba' sample. The alkaline-extracted xylan using 9.0% NaOH amounted to 34.35±1.02% of the total dry weight of xylan, indicating the optimal yield. As the concentration of NaOH increased above 9%, the amount of xylan gradually decreased. The amount of xylan decreased to 19.68 ± 0.33 when 15% NaOH was used (Gosh *et al.,* 2021).

Production of Xylanase from *Aspergillus flavus*

Solid State fermentation (SSF) was used for the production of xylanase on a defined medium containing 1.0g of xylan extracted from 'Goruba' fruit waste, and three (3) three-step purification procedure was adopted as shown in Table 3. The total crude xylanase was 362.3 mg/mL, and ammonium sulfate precipitation was 41.17 mg/mL, 9.480 mg/mL after DEAE Sepharose treatment, while 2.860 mg/mL was obtained when eluted with Sephadex gel. The Total xylanase activity was 2829 IU and retained 96.74% yield of the activity after ammonium sulphate fractionation. The enzyme was finally purified to 22.59 times with a yield of 22.69% and a specific activity of 224.48 IU/mg.

Xylanase Assay

The Sugars and uronic acid composition generated during hydrolysis of the xylan obtained from Goruba' by *Aspergillus flavus* xylanase is presented in Table 4. The results obtained are lower than those reported by Ozili (2023). They produced Xylooligosaccharides from tobacco stalk, cotton stalk, sunflower stalk, and wheat straw using xylanase produced from T. longibrachiatum and *A. niger*. According to their result, cotton straw had the highest glucose of 13.5 % and wheat straw produced the highest arabinose (10.8 %), with xylose content in sunflower straw of 86.6 % and Tobacco stalk produced 10.7 % of uronic acid

Characterization of Xylooligosaccharides

The High-Performance Liquid Chromatography (HPLC) fractional composition of Xylooligosaccharides generated from the xylan obtained from "Goruba" is given on Table 5. The action of *A. flavus* xylanase on the xylan produced mainly X2, X3, and X4 with a small amount of X1, X5, X6, and >X_6

Table 1: Lignocellulosic Composition of 'Goruba' fruit waste sample (%)

S/N	Sample	Cellulose	Lignin	Hemicellulose
1	Goruba fruit waste (X± SD)	23.62±0.73	31.13±0.22	32.78±0.12

Key: X=Mean and SD=Standard deviation

Table 2: Xylan from 'Goruba' fruit waste.

NaOH concentrations	3% NaOH	6% NaOH	9% NaOH	12% NaOH	15% NaOH
Goruba Waste Xylan (X± SD)	9.16±1.12	12.05±1.21	34.35±1.02	24.40±0.23	19.68±0.33

Key: X=Mean and SD=Standard deviation

Table 3: Three steps Purification of Xylanase from *Aspergillus flavus*

Purification	Total protein (mg)	Total activity (1U)	Specific activity (1U/mg)	Fold	Yield (%)
Crude Enzyme	362.3	2829	7.8100	1.000	100.0
Ammonium Sulphate	41.17	2737	66.480	8.512	96.74
DEAE Sepharose A-50	9.480	1672	176.37	22.59	61.09
Sephadex gel	2.860	642.0	224.48	28.74	22.69

Values are the mean of triplicate determinations

Table 4: Sugars and Uronic acid of 'Goruba' Xylan Generated by *A. flavus* xylanase

Xylan composition	Arabinose	Xylose	Glucose	Uronic acid
Fractions (%).(X± SD)	8.97±1.31	69.96±0.96	7.210±0.74	13.79±0.62

Key: X=Mean and SD=Standard deviation

Table 5: HPLC Fractions of Xylooligosaccharides in mg/mL

Sample/ Fractions	X_1	X_2	X_3	X_4	X_5	X_6	>X_6
GW (X± SD)	0.323	0.946	0.603	0.356	0.184	0.162	0.406

KEY: GW=Goruba waste. X_1=xylose, X_2=xylobiose, X_3=xylotiose, X_4=xylotetrose, X_5=xylopentose, X_6=xylohexose,

Effect of Temperature and pH on xylanase Activity

Table 6 provides the effect of temperature on the conversion of xylan obtained from 'Goruba' to XOS by Aspergillus *flavus* xylanase. The optimum temperature for enzyme activity was 50°C, at which it retained 100% of its relative activity. Ozili (2023) and many others have investigated the effect of temperature on the activities of xylanases, reporting an optimal temperature of 50°C. On the other hand, Gautério *et al.* (2021) reported the highest xylanase activity of T. reesei at 60°C. The effect of pH on enzyme activity is presented in Table 7. The optimal pH was 6.0, which gradually decreased above or below this value. Similarly, Alokika and Singh (2019) reported that the optimum pH for xylanases produced by many

Aspergillus and *Penicillium species* ranges from 5.0 to 6.0. Barbieri *et al.* (2022) reported an optimum activity of *A. fumigatus* xylanase at pH 8, which is higher than the 6.0 obtained in this study.

Table 6: Effect of Temperature on Activity of Enzymes on 'Goruba' Xylan

Temp. (°C)	*A. flavus* Xylanase Activity (IU) (X± SD)	*A. flavus* Xylanase Relative Activity (IU)
30	6.87 ±0.53	32. 87 ±0.13
40	14.64±0.53	47.62±0.05
50	29.33±0.48	100.0±0.48
60	22.43±0.94	75.81±0.42
70	10.32±0.82	35.07±0.39
80	3.070±0.61	10.43±0.63
90	0.480±0.72	1.631±0.70

Readings are the standard deviation of triplicate determinations (n=3)

Table 7: Effect of pH on Activity of Xylanase on 'Goruba' Xylan

pH	*A. flavus* Xylanase Activity. (X± SD)	*A. flavus* Xylanase Relative Activity
3	20.18±0.92	73.36±0.11
4	22.42±0.87	81.50±0.46
5	26.67±1.02	89.68±0.32
6	27.51±0.27	100.0±0.52
7	26.92±0.19	96.32±0.43
8	23.13±0.68	85.19±0.08
9	16.74±0.73	59.26±0.17

Readings are standard deviation of triplicate determinations (n=3)

Table 8: ANOVA Analysis Results for Response Surface Quadratic Model

Source	Sum of Square	df	Mean Square	F-value	P-value	
Model	185.20	20	9.26	42.94	< 0.0001	Significant
A-Temperature	25.10	1	25.10	116.41	< 0.0001	
B-pH	1.02	1	1.02	4.71	0.0397	
C-Time	13.29	1	13.29	61.62	< 0.0001	
D-Enzyme Dose	5.71	1	5.71	26.49	< 0.0001	
E-Substrate Dose	51.59	1	51.59	239.25	< 0.0001	
AB	4.24	1	4.24	19.68	0.0002	
AC	25.91	1	25.91	120.15	< 0.0001	
AD	0.2304	1	0.2304	1.07	0.3112	
AE	1.25	1	1.25	5.82	0.0235	
BC	6.68	1	6.68	30.99	< 0.0001	
BD	0.0042	1	0.0042	0.0196	0.8898	
BE	3.82	1	3.82	17.73	0.0003	
CD	6.13	1	6.13	28.41	< 0.0001	
CE	7.73	1	7.73	35.84	< 0.0001	
DE	0.1600	1	0.1600	0.7420	0.3972	
A^2	14.35	1	14.35	66.57	< 0.0001	
B^2	.1328	1	0.1328	0.6157	0.4400	
C^2	10.51	1	10.51	48.75	< 0.0001	
D^2	0.3234	1	0.3234	1.50	0.2321	
E^2	13.86	1	13.86	64.26	< 0.0001	
Residual	5.39	25	0.2156			
Lack of Fit	4.67	20	0.2335	1.62	0.3118	Not significant
Pure Error	0.7205	5	0.1441			
Cor Total	190.57	45				
S. D					0.4644	
Mean					21.700	
CV					2.1400	
R^2					0.9717	
Adjusted R^2					0.9491	
Predicted R^2					0.9365	
Adeq Precision					27.316	

Key: C.V = Coefficient of Variation, S.D = Standard Deviation, R^2 = regression coefficient

Table 9: Comparison of growth of *Lactobacillus acidophilus* on different media (cfu/g)

S/N	Samples	No. of colonies	Bacterial load (cfu/g)
3	Modified medium with Goruba XOS (carbon source)	18	1800 or 1.8 x 10^3
4	MRS media	22	2200or 2.2 x 10^3
5	Media with only agar agar as carbon source (Control)	3	3000 or 3.0 x 10^3

Readings are mean of triplicate determinations

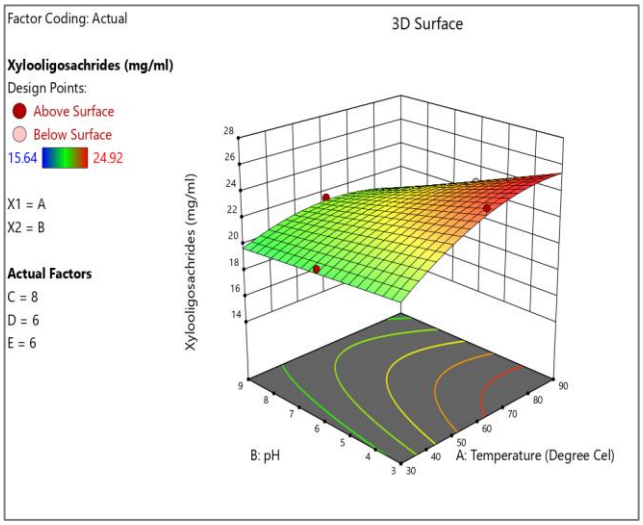

Figure .1a: Temperature and pH interaction

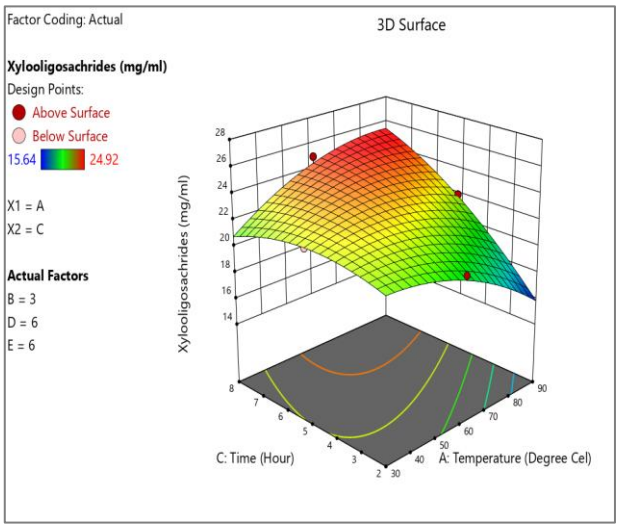

Figure .1b: Temperature and time interaction

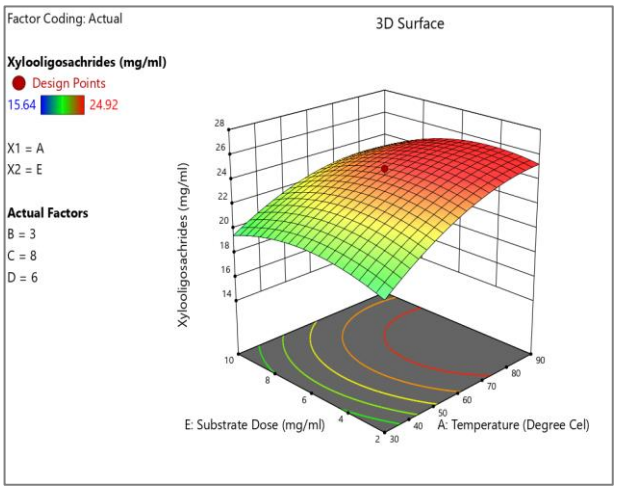

Figure .1c: Substrate dose and temperature interaction.

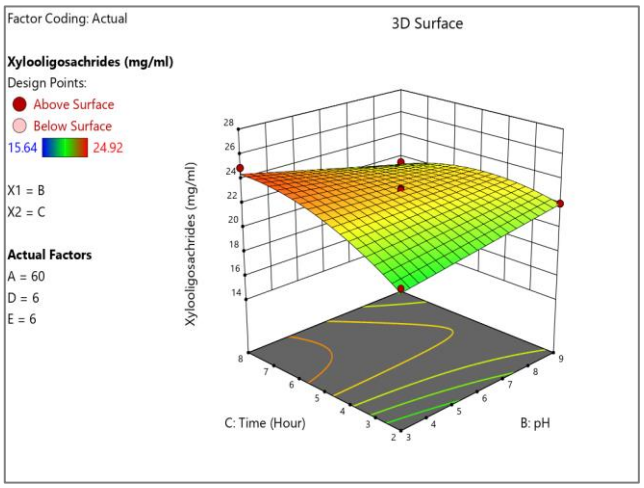

Figure .1d: Time and pH interaction

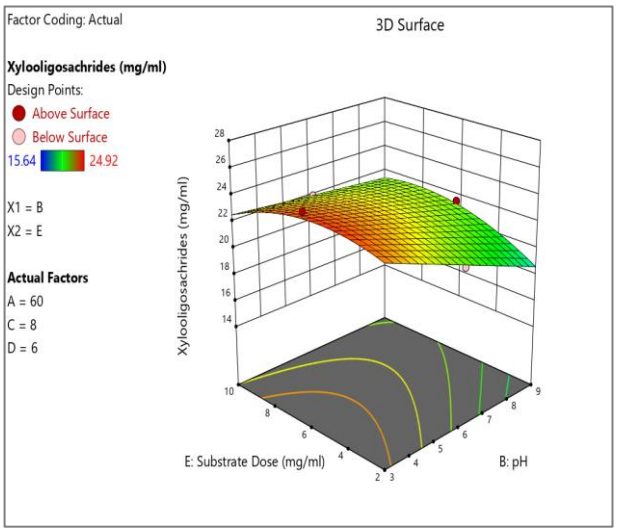

Figure 1e: Substrate dose and pH interaction

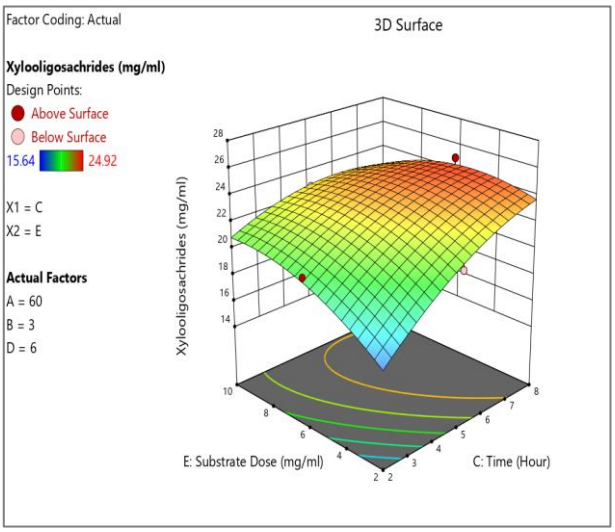

Figure 1f: Substrate dose and time interaction

Figure 1: Response surface of interaction effect plots of independent variables

Growth of *Lactobacillus acidophilus* on different media

Table 9 shows the growth of *Lactobacillus acidophilus* on modified medium with Goruba XOS as carbon source, MRS media and on media formulated with only agar agar as carbon source (Control). Highest growth was observed on MRS media.

Statistical Analysis and Optimization

Table 8 shows the analysis of variance (ANOVA) for the conversion of xylan to XOS by *Aspergillus flavus* xylanase. The table examined the effect of individuals and the interaction of the independent variables on the response variable (XOS), as well as the significance and fitness of the model, comparing quadratic versus two-factor interactions (2FI). The XOS was analyzed using a conversion regression equation in terms of the coded factor as:

Xylooligosaccharides = + 22.99 - 1.26 *A -0.251 *B +0.951 *C - 0.556 *D +1.80 *E - 1.03 *AB +2.54 *AC - 0.24 *AD - 0.56 *AE - 1.29 *BC - 0.0325 *BD +0.9775 *BE - 1.40 *CD -1.39 *CE -0.200 *DE - 1.27 *A^2 +0.1369 *B^2 - 1.14 *C^2 - 2331 *D^2 - 1.25 *E^2

The model was significant (P-value < 0.0001) and had an F-value of 42.94. On the table, the independent variables that influenced the hydrolysis were: Substrate concentration (239.25) E >Temperature (116.41) A > time (61.62) C > Enzyme dose (26.49) D and pH (4.71) B. Time and substrate interaction (CE) had the highest influence on the conversion of xylan, which is supported by the 3D surface plot (Fig. 1a, 1b, 1c, 1d, 1e & 1f).

DISCUSSION

Prebiotic xylooligosaccharides are reported to be effective in managing certain noncommunicable diseases (NCDs), such as type II diabetes, High blood pressure, and high cholesterol (Valladares-Diestra *et al.*, 2023). NCDs including cardiovascular diseases, hypertension, diabetes, and cancers which are responsible for most deaths globally with over 55% occur in low- and middle-income countries (Berríos-Torres *et al.*,2017; WHO,2023) In Nigeria, the diseases are accounted for over 30% of all death due to genetic, lifestyle, and socioeconomic reasons (Idris *et al.,* 2020). Recently, the food industry is developing different types of functional foods, such as prebiotics (Xylooligosaccharides), to curtail the incidence of these ailments with greater health benefits. Xylooligosaccharides (XOS) are nondigestible oligosaccharides obtained from xylan with high prebiotic potential (Valladares-Diestra *et al.*, 2023). Xylan is used for the production of XOS due to its susceptibility to hydrolysis by xylanase (Valladares-Diestra *et al.*, 2023). The amount of xylan obtained from this study indicates that 'Goruba' fruit waste is a good source of XOS when compared with the results of many researchers. For instance, Aachary et al. (2019) reported 10.20 mg/mL of xylooligosaccharides from corncob using *A. oryzae* xylanase. Additionally, Valladares-Diestra *et al.*, (2023) used *A. brasiliensis* xylanase to produce XOS from rice husk and obtained 18.35 mg/mL. Similarly, Nakamichi *et al.* (2019) reported 6.73 ± 0.23 mg/mL of XOS from partially purified *A. foetidus* xylanase. The potency of the XOS produced from 'Goruba' fruit waste on the growth of *Lactobacillus acidophilus* revealed that MRS agar media recorded the highest number of colonies, followed by modified media containing 'Goruba' XOS as the only source of carbon. This result suggests that XOS is capable of supporting the growth of *Lactobacillus acidophilus*, which is considered one class of probiotics. The potency of the XOS on the growth of *Lactobacillus acidophilus* has established that it is suitable for large scale production of the prebiotic-XOS from the local biomass.

Goruba is widely available across the northern part of Nigeria and has not been properly assessed as a feedstock for the production of valuable products. Many of the fruit wastes are usually left to rot in the field, resulting in a huge economic loss. The plant biomasses can be used to produce XOS, thereby adding value to the biomass. Xylanase obtained from *A. flavus* for the production of XOS exhibited favorable properties, such as pH and temperature (Asad et al., 2013). Murthy *et al.* (2012) purified xylanase from *Aspergillus fumigatus* and reported an optimum temperature of 50°C and a pH of 5.0, which agrees with the 50 °C obtained in this study. According to Barbieri *et al.* (2019), Aspergillus xylanase activity can be active between 50 °C and 65 °C with an average half-life of 2 to 6 hours. The results from this work agree with those reported by Alokika et al. (2019), which indicate that the optimum pH for xylanases of many Aspergillus and Penicillium species ranges from 5.0 to 6.0. Chen *et al.* (2019) reported the optimal temperature of 55°C and pH 7.5 for xylanase from *Aspergillus flavus*. The result is higher than the one obtained in this study. Statistically, all five independent variables had a significant effect on the conversion of Goruba xylan to XOS, except for pH, which could be due to experimental error. Gautério. *et al* (2022) also reported the least influence of pH on *Aureobasidium pullulans* xylanase. The regression coefficient (R^2) value of 0.9717 indicated good precision and adequacy of the model. The adjusted R^2 value of 0.9491 was in close agreement with the predicted R^2 value of 0.9365 (a difference of less than 0.2), indicating a good relationship among the variables. The low coefficient of variation (CV) of 2.14% and standard deviation of 0.9752 indicated good precision and reliability of the experimental results. The lack of significant fits indicated that the model was accurate with minimal error (Forsan *et al.,* 2023). The numerical and optimal conditions suggested the yield of 26.05 mg/ml at a temperature of 52.54 °C, pH of 7.90, at 2 hours, with an enzyme dose of 2 mg/ml and a substrate concentration of 8.50 mg/ml

CONCLUSION

The high quantity of xylan and subsequent amount of xylooligosaccharides produced from 'Goruba' fruit waste indicated that it is a good raw material for XOS. The numerical optimized conditions of 'Goruba' xylan to XOS by *Aspergillus flavus* xylanase suggested the yield of 26.05 mg/mL at temperature (52.4℃), pH (7.90) at 2 hours, enzyme dose (2 mg/mL) and substrate concentration

(8.50 mg/ML) shown a higher value than the experimental result of 24.93 mg/mL. The use of these optimal conditions for production is therefore recommended for achieving higher yields. Utilizing biomass may add value to it, leading to a more sustainable agricultural system and promoting green chemistry and bio-refinery concepts. The process is environmentally friendly since the enzymatic hydrolytic method reduces the dependence on hazardous synthetic chemicals. XOS production from biomass will also help boost agribusinesses, contributing to local economies and creating opportunities in biomass collection and processing.

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