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ORIGINAL RESEARCH ARTICLE

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

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ABSTRACT

Goruba (*Hyphaene thebaica*) fruit waste is an underutilized, xylan-rich residue that can be enzymatically converted into xylooligosaccharides (XOS). XOS are non-digestible prebiotic oligosaccharides that function as soluble dietary fiber by supporting beneficial gut microbes. The aim of this research was to valorize 'Goruba' (*Hyphaene thebaica*) fruit waste for XOS production. Xylan was obtained from 'Goruba' sample using alkaline extraction. *Aspergillus flavus* was used to synthesize xylanase enzyme from xylan medium under solid state fermentation (SSF) and the xylanase was assayed using DNS method. Hydrolysis of xylan to XOS was carried using the fungal xylanase. Produced XOS was analyzed using HPLC. Production of XOS was optimized using Response Surface Method (RSM) approach. One way ANOVA ($p < 0.05$), mean ± SD and numerical optimization were adopted to analyze the data. Highest xylan yield was 34.35±1.02% with 9.0% NaOH. Total protein in the crude extract was 362.3 mg with total activity 2,829 U and specific activity 7.81 U/mg. Xylanase activity peaked at 22.33 U/mL at 50 °C, pH 6.0. The HPLC fractions (mg/mL) were xylose 2.43, xylobiose 8.65, xylotriose 5.22, xylotetraose 2.75, xylopentaose 1.03, xylohexaose 0.81, and DP > 6 = 4.03, totaling 24.92 mg/mL. Ammonium sulphate precipitation gave protein (41.17 mg), total activity (2737 U), specific activity (66.48U/mg) and 96.74% yield. Numerical optimized XOS was 26.05 mg/mL at 52°C, pH (7.90), at 2 hours, enzyme dose (2 U/mL) and substrate dose (8.50 mg/mL). This study has established that the 'Goruba' fruit waste is a good source of prebiotics-XOS and can be used for large-scale production due to its affordability and availability.

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INTRODUCTION

Xylooligosaccharides (XOS) is a rapidly growing class of oligosaccharides with broad range of applications as prebiotics, used in cosmetics, agriculture, medicines, and in food industry (Coelho *et al*., 2025). XOS are classified as complex sugars the body cannot digest, they are mainly derived from hydrolysis of xylan, a component of lignocellulosic materials found in plant cell walls and contains xylose as monomers with unit chain of 2 to 10 (Fuso *et al*., 2022). As a prebiotic and as functional food, it offers many health and nutritional benefits. Small trials suggest that it may lower blood pressure and cholesterol, promoting gastrointestinal well-being, exhibiting anti-cancer properties and regulating blood sugar (Nogueira-Prieto *et al*., 2025). Advancement in healthcare sector has led to an increasing recognition of XOS over other functional foods and prebiotics due to its 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. (Azzouz *et al.,* 2021; Khizar *et al*., 2024). Deficiencies associated from lack of taking prebiotic fiber include digestive disorder, weakness of the immune system, 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, optimization of raw materials and production conditions from local raw materials (Sun *et al*., 2024). Despite extensive studies on lignocellulosic residues such as wheat straw, corn cob, and sugarcane bagasse for XOS production using microbial xylanases, the valorization of ("Goruba") fruit waste remains virtually unexplored. 'Goruba', widely available across sub-Saharan Africa, has been shown to contain substantial xylan but has not been systematically assessed as a feedstock for XOS production using A. *flavus* xylanase. 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

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resulting XOS. Addressing these gaps is essential to establish that '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. Pure culture of *Aspergillus flavus* was obtained at Department of Microbiology, Modibbo Adama University, Yola. Adamawa State. Pulp of ripened and dried 'Goruba' fruit was removed, washed and boiled with potable water to obtain the chaff. The chaff was drained and oven dried at 55°C to constant weight before milling into flour using roller mills. The flour was sieved with 0.02 mm sieve and stored in a cool and dry place plastic container (AOAC, 2006).

Determination of Hemicellulose

Two (2) g of the 'Goruba' 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 filtering on tared Gooch crucible. The residue was thoroughly washed with acetone and finally with boiling water before drying at 100 °C and weighed to get 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 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 out and oven dried to constant weight at 60 °C (Lehuedé, *et al.*, 2024).

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

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 for seven days at 28 ± 2°C. The crude enzyme was harvested by adding 200 ml of distilled water with addition of minimum amount of sodium citrate buffer (50 mM, pH 5.3) and homogenized thoroughly. The slurry was filtered through cheese cloth and centrifuged at 4000 rpm for 20 min at 4°C. The supernatant was again filtered through Whatman No.1 filter paper and the filtrate to obtain the crude enzyme (Asad *et al.*, 2013).

Estimation of Xylanase Total Protein.

Bradford method was used to determine the crude total protein as described by (Srivastava *et al.*, 2025). The Bradford reagent was prepared by dissolving 100 mg of Coomassie Brilliant Blue G250 in 50 mL of 95% ethanol and 100 mL of 85% phosphoric acid .The solution was filtered before adding 100 mL of glycerol before solution was adjusted with distilled water to 1000 mL. The reagent was allowed to stand for 24 hours before use. One (1.0) mg of Bovine serum albumin (BSA)/mL was prepared as protein standard stock solution. Different standard protein solutions were prepared to make 0.2, 0.4, 0.6, 0.8, and 1.0 mL into series of test tubes and their volume adjusted to 1.0 mL. To other two separate test tubes, 0.2 mL of the test sample was pipetted and the volume adjusted to 1.0 mL. One (1.0) mL of water was pipetted to another test tube mark as a blank reference. To all the test tubes, 5.0 mL of the Coomassie Brilliant Blue reagent was added and thoroughly mix by vortexing. The solutions ware incubated for 10-30 minutes before measuring the absorbance at 595 nm. Standard curve graph was plotted from the absorbance of the standard BSA against their known concentrations. And the graph was used to determine the protein content of the sample by extrapolation (Srivastava *et al.*, 2025)

Three Step Purification of Crude Xylanase

Ammonium sulphate precipitation

Protein precipitation by salting out technique using ammonium sulphate ($(NH_4)_2SO_4$) 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 pH of 8.60 before dialyzing against the same buffer for 24h using cellulose tubing (molecular weight cut-off 13kDa and Himedia LA393-10MT (Kamble, *et al.*, 2012).

Ion Exchange Chromatography.

DEAE Sephadex A-50 ion exchange chromatography was used to further purify the dialyzed enzyme after ammonium sulphate precipitation. Two (2ml) of dialyzed xylanase was loaded onto an anion exchange DEAE-Sephadex A-50 (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 flow rate of 1mL/min carried out at 4°C and stored at 4 °C (Lu *et al.*, 2008)

Sephadex G-100 gel filtration

The purified enzyme obtained after ion exchange chromatography was further purified with Sephadex G-100 packed in to 2.6 by 50 cm column, equilibrated with 50mM citrate buffer (pH 5.3). The xylanase sample was introduced into the column, washed with the same buffer before eluting with 0.1 M of KCl solution at a flow rate of 10 mL//h.. The xylanase was further concentrated by lyophilization and used as partially purified enzyme (Lu *et al.*, 2008)

Xylanase Assay

The xylanase activity was quantified based on reducing sugar generated by 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 they were heated with 3,5-dinitrosalicylic acid to form a red-brown solution. The optical density (OD) of the coloured complex was determined spectrophotometrically at 540 nm. The graph of concentration against the optical density was plotted using MS Excel and the amount of xylose in the sample was deduced by extrapolating the equivalent concentration of the sample on the X axis and multiply by 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 minutes under the assay condition (Mughal *et al*, 2020). Similar procedure was followed to obtain the concentration of glucose at 505 nm; galactose was determined using the DNS reagent in the presence of alkaline solution at 540nm while arabinose was determined at 660nm. The concentration of uronic acid was estimated by subtracting the concentration of glucose, galactose and arabinose from 100 (AOAC,2006)

Effect of Temperature and pH on Xylanase Activity

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

Hydrolysis of Xylan to Xylooligosaccharides

Goruba xylan pellets were hydrolyzed to xylooligosaccharides using *Aspergillus flavus* xylanase in sodium citrate enzyme buffer solution. The process involved varying the pH from 3, 4, 5, 6, 7, 8 and 9 and temperature from 30, 40, 50, 60, 70, 80 and 90°C, and varying the enzyme dose of 2U, 4U, 6U, 8U, and 10U, where (U = 1.0 µmol/min). 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 taken and analyzed (Aachary and Prapulla 2019).

Prebiotic potency of XOS on *Lactobacillus acidophilus*

The prebiotic potency of XOS was assessed by enumerating the growth of *Lactobacillus acidophilus* on MRS media and media modified with 'Goruba' XOS. The MRS media contained the following (g/L): 10 g proteose peptone, 5 g yeast extract, 10 glucose, 1 g Tween 80, 2.0 g ammonium citrate, 5 g sodium acetate, 0.1 g $MgSO_4$, 0.05 g $MnSO_4$, 2 g K_2HPO_4 and 12 g agar The pH was adjusted to 6.5 ± 0.2 (HiMedia laboratories, 2023 ;Tian *et al* ., 2024).In the modified media, Dextrose (glucose) was replaced with 10 g of XOS obtained from 'Goruba' . The pure *L. acidophilus* samples were mixed with sterile saline at a ratio of 1/9 dilution; subsequently, 0.1 mL of 10^{-1} to 10^{-5} serial dilutions were anaerobically cultured on MRS agar and on modified MRS agar and incubated at 37°C. After 48 h, the number of colony-forming units (CFUs) was counted (Kumari *et al.*, 2024; Yahyaoui *et al.*, 2025).

Characterization of Xylooligosaccharides (XOS)

The concentration and fractions of xylooligosaccharides (XOS) from hydrolyzed xylan were determined using a High-Performance Liquid Chromatography (HPLC) system (Agilent, USA). The system was equipped with a refractive index detector (PerkinElmer Series 200) and a Shodex Sugar KS-802 packed column (8 mm ID x 300 mm, F6378020), with the column maintained at 65 °C. Samples were eluted with deionized water at a flow rate of 0.5-1.0 mL/min. The limit of quantification (LOQ) for the method was 0.7 mg/mL, as established by AOAC (2006). A 5 mL sample was prepared at a concentration of 0.1 g/100 mL, filtered through a 0.2 m membrane (Minigen USA- MG-25020PVDF), and a 20 L aliquot was injected manually. XOS was quantified by comparing average peak areas to those of standard XOS solutions (xylose, xylobiose, xylotriose, xylotetrose, and xylopentose). Final concentrations were reported in mg/mL, based on methods outlined by Ristović et al. (2024).

Data Analysis and optimization of XOS production

All data used for this study were replicated. Standard deviation, Fit statistics and one way ANOVA were adopted to analyze the data using design expert version 13. Five independent variables and one response were studied using Response Surface and Box Benkhen 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), enzyme and substrate dose ranged from 2,3,4,5,6,7,8,9, and 10 mg (Dhaver *et al.*, 2023). The precision of experimental data was further studied using regression model (R^2), adjusted regression square (Adj-R^2) and 3D 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 sample

Table 1 shows the lignocellulosic composition of 'Goruba' fruit waste sample which shows appreciable amount of hemicellulose when compared with other literature.

Quantification of Xylan

Table 2 show the result of xylan obtained from 'Goruba' sample. The Alkaline extracted xylan using 9.0% of NaOH amounted to 34.35±1.02% of the total dry weight of xylan and indicated 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% of NaOH was used.

Production of Xylanase from *Aspergillus flavus*

Solid State fermentation (SSF) was used for production of xylanase on a defined media containing xylan extracted from 'Goruba' fruit waste and three (3) steps purification procedures as shown on Table 3. The total crude xylanase was 362.3 mg and ammonium sulphate precipitation were 41.17mg, 9.480 mg after DEAE Sepharose elution while 2.860 mg was obtained when eluted with Sephadex gel. The total xylanase activity was 2829 U and retained 96.74% yield of activity after ammonium sulphate fractionation. The enzyme was finally purified to 22.59 folds with a yield of 22.69% and specific activity of 224.48U/mg.

Xylanase Assay using Dinitrosalicylic acid (DNS) method

The Sugars and uronic acid composition generated during hydrolysis of the xylan obtained from Goruba' by *A. flavus* xylanase is presented on Table 4.

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 small amount of X1, X5, X6 and >X_6 (>6 =Degree of polymerization (DP) of xylose units greater than 6)

Effect of Temperature and pH on xylanase Activity

Table 6 shows the effect of temperature on the conversion on xylan obtained from 'Goruba' to XOS by *Aspergillus flavus* xylanase. The optimum temperature for the enzyme activity was 50°C and retained 100 % of the relative activity. The effect of pH on the enzyme activity is presented on Table 7. The optimal pH was 6.0 which gradually decreased above or below 6.0.

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 (U)	Specific activity (U/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 mean of triplicate determinations. Notes: (U= 1.0 µmol/min)

Table 4: Fractions obtained from hydrolyzed 'Goruba' xylan by *A. flavus* xylanase

Hydrolyzed Xylan	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 XOS produced by *Aspergillus flavus* xylanase (mg/mL)

Sample/ Fractions	X_1	X_2	X_3	X_4	X_5	X_6	>X_6
Goruba xylan (XOS = 24.92)	2.43	8.65	5.22	2.75	1.03	0.81	4.03

KEY: X_1=xylose, X_2=xylobiose, X_3=xylotriose, X_4=xylotetraose, X_5=xylopentaose, X_6=xylohexaose, >6 =Degree of polymerization of xylose units greater than 6

Growth of *L. acidophilus* on MRS and modified media with XOS

Table 8 shows the growth of *Lactobacillus acidophilus* on commercial MRS Agar and modified Agar containing 'Goruba' XOS as carbon source. Higher (34 colonies) number of colonies was obtained on MRS Agar while modified MRS Agar with 'Goruba' XOS produced 30 colonies.

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

Temp.(°C)	*A. flavus* Xylanase Activity (U) (X± SD)	*A. flavus* Xylanase Relative Activity (%)
30	6.87 ±0.53	23.40 ±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 standard deviation of triplicate determinations (n=3) (U= 1.0 µmol/min)

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

pH	*A. flavus* Xylanase Activity (U). (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	96.93±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) (U = 1.0 µmol/min)

Table 8: Growth of *L. acidophilus* on MRS and modified media with XOS

S/N	Samples	No.of colonies	Bacterial load (cfu/g)
1	Modified MRS Agar ('Goruba' XOS as carbon source)	30	3.0×10^3
2	MRS Agar	34	3.4×10^3

Readings are average of triplicate determinations

Table 9: 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	0.397	
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

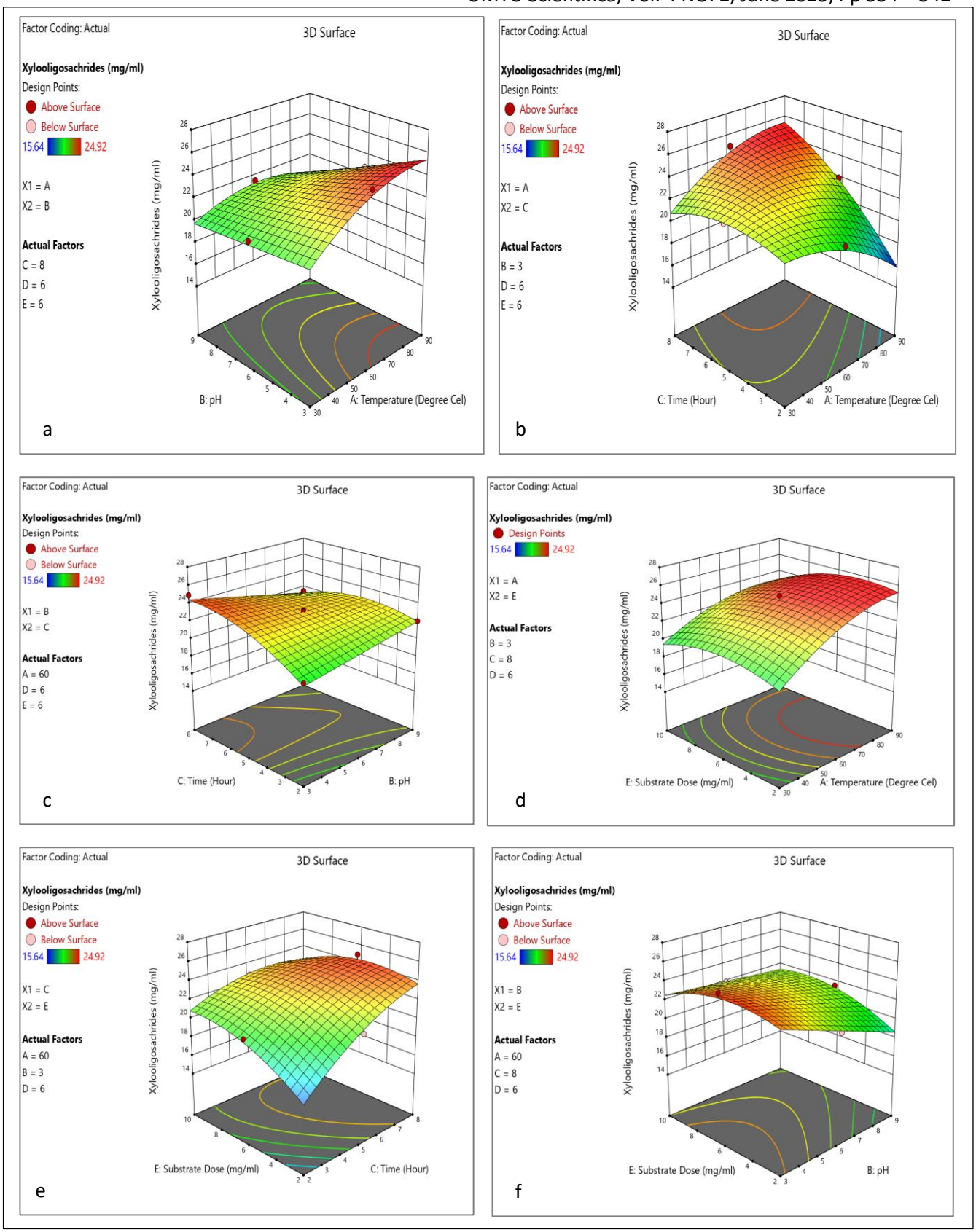

Figure 1: Response surface of interaction effect plots of independent variables
a: Temperature and pH interaction; **b**: Temperature and time interaction; **c**: Time and pH interaction; **d**: Substrate dose and temperature. Interaction; **e**: Substrate dose and time interaction; **f**: Substrate dose and pH interaction

Statistical Analysis and Optimization

Table 9 show the analysis of variance (ANOVA) for the conversion of xylan to XOS by *Aspergillus flavus* xylanase. The table studied the effect of individuals and interaction of the independent variables on the response variable (XOS) as well as the significance and fitness of the model as suggested by quadratic versus two factor interactions (2FI). The XOS was analyzed using 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 – 0.231 *D^2 - 1.25 *E^2 .The model was significant (P-value of less than 0.0001) and 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 3D surface plot (Figure 1e).

DISCUSSION

This study demonstrates that Goruba **(***Hyphaene thebaica***)** fruit waste is a viable**,** xylan-rich feedstock for xylooligosaccharide (XOS) production. We obtained 32.78 **%** hemicellulose and 34.35 % xylan, produced an *A. flavus* xylanase whose activity peaked at 22.33 U/mL at 50 °C, pH 6.0**,** and achieved a total XOS of 24.92 mg/mL. Many authors have reported the different amount of XOS from different plant biomass, for instance, Ali *et al.,* 2025 reported 10.23 mg/mL from sugarcane bagasse and 17.23 mg/mL from rice husk. Aachary and Prapulla (2019) also reported 10.20 mg/mL from corncob using *A. Oryzae* xylanase. Also, Kumari *et al*., (2024) reported the use of *A. brasiliensis* xylanase to produce XOS from rice husk and obtained 18.35 mg/mL yield. Similarly, Khat-udomkiri *et al*., (2020) reported 6.73 ± 0.23 mg/mL from rice husk using partially purified *A. foetidus* xylanase. Response-surface analysis identified substrate dose and temperature as the strongest positive drivers of XOS, while pH had insignificant effect (p = 0.397), and predicted numerical optimal XOS value of 26.05 mg/mL. These optima conditions are consistent with reported Aspergillus xylanase behavior (≈50–65 °C; pH 5–6) (Barbieri, *et al.,* 2019; Alokika *et al.* 2019; Chen *et al.* 2019; Murthy and Naidu, 2012). Compared with reported totals from bagasse and rice husk, our XOS concentration is higher; however, differences in substrate composition, enzyme preparation, and analytical methods may contribute to these gaps.

Statistically, all the five independent variables played significant effect on the conversion of Goruba xylan to XOS except pH which could be due to experimental error. Gautério. *et al* (2021) also reported the least influence of pH on *Aureobasidium pullulans* xylanase. The regression coefficient (R^2) of 0.9717 showed a good precision and adequacy of the experimental data. Additionally, the adjusted R^2 value of 0.9491 was in close agreement with predicted R^2 value of 0.9365 which showed a good relationship among the variables. The low coefficient of variation (CV) of 2.14 % and standard deviation (0.4644) indicated a good precision and reliability of the experimental results. The non-significant lack of fits indicated that the model was good with less error (Forsan *et al.,* 2023). The numerical and optimal conditions suggested the yield of 26.05 mg/mL at temperature of 52.54°C, pH of 7.90, at 2 hours, with enzyme dose of 2 U/mL and substrate concentration of 8.50 mg/mL which is higher than the 24.92 mg/mL gotten from this experiment. Chinbat *et al*., (2024), tested the potency of XOS obtained from wheat bran on probiotic *B. adolescentis* and *L. brevis* and reported the utilization of the XOS as source of carbon. On the other hand, pathogenic *E. coli* did not show any growth on the modified media containing the wheat bran XOS, this indicate that XOS are selective to only probiotics (Kumari *et al*., 2024). The 30 number of colonies obtained from modified MRS Agar with 'Goruba' XOS as carbon source in this study has established that prebiotic has potentials of supporting the growth of probiotic *lactobacillus acidophilus*.Prebiotic Xylooligosaccharides (XOS) may be effective in managing some non-communicable diseases (NCDs) such as type II diabetes, High blood pressure, bad cholesterol and cancer (Kumari *et al*., 2024). NCDs are responsible for most deaths among the aged people globally especially in low and middle-income societies. In Nigeria, the diseases are accounted for over 30% of death in adults due to genetic, lifestyle, and socioeconomic reasons (Obada et al., 2024). Recently, food industries are developing different functional foods such as prebiotic (XOS) to curtail these ailments. Xylooligosaccharides have higher prebiotic potential when compared with other prebiotics selectivity or specificity to probiotics (Kumari *et al*., 2024). Xylan is used for production of XOS due to its susceptibility to hydrolysis by xylanase (Kaprelyants *et al*., 2017)

The biomass is widely available across the northern part of Nigeria and is usually left as a waste. This can be explored to produce industrially important substances like XOS.

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 U/mL) and substrate concentration (8.50 mg/mL) shown a higher value than the experimental result of 24.92 mg/mL. The use of these optimal conditions for the production is therefore recommended for higher yield. Utilizing the biomass may add value to it, leading to more sustainable agricultural system, promote green chemistry and bio-refinery concepts. The process is environmentally friendly since enzymatic hydrolytic method reduces the dependence on hazardous synthetic chemicals. XOS production from the biomass will also help to boost agribusinesses and create opportunity in biomass collection and processing.

REFERENCES

Aachary, A., & Prapulla, S. (2019). Xylooligosaccharides (XOS) as an emerging prebiotic: Microbial synthesis, utilization, structural characterization, bioactive properties, and applications. *Food Science and Food Safety, 10*(1), 2–16. [Crossref]

Ali, K., Niaz, N., Waseem, M., Ashraf, W., Hussain, M., Khalid, M. U., ... & Khan, I. M. (2025).

Xylooligosaccharides: A comprehensive review of production, purification, characterization, and quantification. *Food Research International*, 181, 115631. [Crossref]

Alokika, & Singh, B. (2019). Production, characteristics, and biotechnological applications of microbial xylanases. *International Journal of Biological Macromolecules*, *103*, (pp8763–8784) [Crossref]

AOAC, (2006). Official Methods of Analysis, 18th Ed. Association of Official Analytical Chemists, Will behington D.C, USA.

Asad, A., Mohommad, A., & Veena-Pande, P. (2013). Purification and characterization of xylanase from *Aspergillus fumigatus* isolated from soil. *African Journal of Biotechnology*. 12 (20) (pp 3049-3057)

Azzouz, Z., Bettache, A., Boucherba, N., Prieto, A., Martinez, J., Benallaoua, S., & de Eugenio, I. (2021). Optimization of β-1, 4-endoxylanase production by an *Aspergillus niger* strain growing on wheat straw and application in xylooligosaccharides production. *Molecules*, *26*(9), (pp 2527) [Crossref]

Barbieri, S., Bento, B., de Oliveira, F., Picheli, P., Dias, M., Masarin, F., & Santos-Ebinuma, C. (2022). Xylanase production by *Talaromyces amestolkiae* for valuing agro-industrial byproducts. *Biotechnology*, *11*(2), (pp15) [Link]

Chen, Y., Xie, Y., Ajuwon, M., Zhong, R., Li, T., Chen, L, & Everaert, N. (2020). Xylo-oligosaccharides, preparation and application to human and animal health: a review. *Frontiers in Nutrition*, 8, 731930. [Crossref]

Chinbat, O., Erdenetsog, P., Tuvshintur, B., Gantumur, A., Burenjargal, M., Chimeddorj, B., & Janlav, M. (2024). In vitro and in vivo investigation of the biological action of xylooligosaccharides derived from industrial waste. *Food Science & Nutrition, 12*(10), 7877–7884. [Crossref]

Coelho, D., Costa, D. F., Barroca, M., Cunha, S. A., Pintado, M. M., Abreu, H., ... & Collins, T. (2025). Simplified, high yielding extraction of xylan/xylooligosaccharides from *Palmaria palmata*: The importance of the algae preservation treatment. *Marine Drugs, 23*(8), 302. [Crossref]

Dhaver, P., Pletschke, B., Sithole, B., & Govinden, R. (2023). Optimization of xylooligosaccharides production by native and recombinant xylanase hydrolysis of chicken feed substrates. *International Journal of Molecular Sciences*, *24*(23), (pp 17110 [Crossref]

Forsan, F., de Freitas, C., Masarin, F., & Brienzo, M. (2023). Optimization XOS production from sugarcane bagasse and leaf using *Aspergillus versicolor* endoxylanase and diluted acid. *Biomass Conversion and Biorefinery*, *13*(4), (pp 3375-3390)

Fuso, A., Rosso, F., Rosso, G., Risso, D., Manera, I., & Caligiani, A. (2022). Production of xylo-oligosaccharides (XOS) of tailored degree of polymerization from acetylated xylans through modelling of enzymatic hydrolysis. *Food Research International, 162,* 112019**.** [Crossref]

Gautério, V., Hübner, T., Ribeiro, R., Ziotti, M., & Kalil, J. (2021). Xylooligosaccharide production with low xylose release using crude xylanase from *Aureobasidium pullulans*: effect of the enzymatic hydrolysis parameters. *Applied Biochemistry and Biotechnology*.194 (pp1-20). [Crossref]

HiMedia Laboratories (2023). *Corporate office: Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane (W) - 400604, India.* HiMedia Laboratories Pvt. Ltd. [Link]

Kamble, R. D., & Jadhav, A. R. (2012). Isolation, purification, and characterization of xylanase produced by a new species of Bacillus in solid state fermentation. *International Journal of Microbiology*, *2012*(1), 683193.

Kefas, M., Yunus, R., Rashid, U., & Taufiq-Yap, H. (2018). Modified sulfonation method for converting carbonized glucose into solid acid catalyst for the esterification of palm fatty acid distillate. *Fuel*, *229*, (pp 68-78). [Crossref]

Khat-udomkiri, N., Toejing, P., Sirilun, S., Chaiyasut, C., & Lailerd, N. (2020). Antihyperglycemic effect of rice husk derived xylooligosaccharides in high-fat diet and low-dose streptozotocin-induced type 2 diabetic rat model. *Food Science & Nutrition, 8*(1), 147–157. [Crossref]

Khizar, A., Fatima, M., Khan, N., & Rashid, M. A. (2024). Xylooligosaccharide supplementation in rice protein concentrate-based diets: A comprehensive analysis of performance and health of *Labeo rohita*. *Journal of Animal Physiology and Animal Nutrition, 108*(5), 1059–1071. [Crossref]

Kumari, K., Nagar, S., Goyal, S., Maan, S., Chugh, V., Kumar, V., & Kharor, N. (2024). Xylooligosaccharide production from lignocellulosic biomass and their health benefits as prebiotics. *Biochemistry Research International, 2024*(1), 6179375. [Crossref]

Lehuedé, L., Henríquez, C., Carú, C., Córdova, A., Mendonça, R. T., & Salazar, O. (2024). Xylan extraction from hardwoods by alkaline pretreatment for xylooligosaccharide production: A detailed fractionation analysis. *Carbohydrate Polymers, 302,* 120381. [Crossref]

Lu, F., Lu, M., Lu, Z., Bie, X., Zhao, H., & Wang, Y. (2008). Purification and characterization of xylanase from Aspergillus ficuum AF-98. *Bioresource technology*, *99*(13), 5938-5941.

Murthy, P. S., & Naidu, M. M. (2012). Production and application of xylanase from Penicillium sp. utilizing coffee by-products. *Food and Bioprocess Technology*, *5*, 657-664.

Nogueira-Prieto, N. M., Vallejo, C. A., Becerra-Fernández, M., & González-Siso, M. I. (2025). A review of the capacity of xylooligosaccharides to modulate gut microbiota and promote health. *Food & Function*. Advance online publication. [Crossref]

Obada, A. A., Airaoje, O. K., Okuneye, A. P., Collins-Dike, J., & Msughter, A. E. (2024). Media role on

the burden of non-communicable diseases in Nigeria. *Clinical Case Reports International, 8,* 1652.

Ristović, M., Stojanović, S., Slavić, M. Š., Dojnov, B., Božić, N., Vujčić, Z., & Margetić, A. (2024). A simple and fast HPLC method for determining the composition of fructooligosaccharides and xylooligosaccharides obtained by fungal enzymes. *Journal of Food Composition and Analysis*, *133*, 106459.

Srivastava, Y., Tripathi, K., & Kumar, N. (2025). Quantitative protein estimation using the Bradford assay: Principles, protocols, and applications. *Biotechnology Lab Techniques: Culture Media, Microscopy, and Microbial Analysis,* 157.

Sun, Q., Patil, P. J., Singh, A. K., Teng, C., Zhou, M., Zhou, Y., & Fan, G. (2024). Optimization of pretreatment and enzymatic hydrolysis coupled with ultrasonication for the production of XOS from corn cob. *Biomass Conversion and Biorefinery*, *14*(1), 1215-1235.

Tian, S., Yang, Z., Yan, F., Xue, X. A., & Lu, J. (2024). Preparation of Xylooligosaccharides from rice husks and their structural characterization, antioxidant activity, and probiotic properties. *International Journal of Biological Macromolecules, 271,* 132575. [Crossref]

Yahyaoui, M. I., Bentouhami, N. E., Moumnassi, S., Elbouzidi, A., Taibi, M., Berraaouan, D., ... & Asehraou, A. (2025). Effect of xylooligosaccharides on the metabolic activity of *Lactiplantibacillus plantarum* S61: Production of bioactive metabolites with antioxidant and antimicrobial properties. *Bacteria, 4*(1), 14. [Crossref]