Tender coconut husk waste as a potential source for extraction of Xylooligosaccharides

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Abstract

The present study investigated the application of alkali and steam to extract xylan from tender coconut husk and then enzymatically hydrolysed to produce Xylooligosaccharides (XOS), which has the application as a prebiotic to improve the gut health. 82% of the xylan was separated from the husk using 20% NaOH and steam treatment for 60 minutes. Enzyme concentration and temperature had a substantial impact on xylose yield while enzyme concentration, temperature, time, and pH had an impact on xylobiose yield.

Hemicelluloses in the husk and the presence of xylan in the extracted material were confirmed by FTIR analysis. 2.834 mg/ml of xylose and 2.21 mg/ml of xylobiose were produced at the optimum conditions of crude xylanase enzyme concentration of 5.0%, a pH of 5.4, a temperature of 65°C, and a 6-hour incubation time. From the present study, it has been observed that tender coconut husk is suitable for extracting xylan and XOS.

Keywords: Alkali treatment, FTIR, Tender Coconut husk, Xylan, Xylose, Xylooligosaccharides.

Introduction

India produces more than 11 million tonnes of coconuts annually, making it the third-largest producer of coconuts in the world, behind Indonesia and the Philippines[10]. The husk accounts for a considerable amount of the fruit, making up about 50% of its mass[31,34]. The components of this husk include 26% water-soluble materials[35], 39.31% cellulose, 16.15% hemicellulose, and 28.48% lignin[32]. Tender coconut husk waste is a substantial waste product generated from the tender coconut vendors and is often discarded as waste in the surrounding environment. Bangalore city of Karnataka generates an estimated 300 tonnes of coconut shells a day[10].

Oligosaccharides serves as intermediates between simple sugars and polysaccharides and hold significant importance in food production, where they are employed as bulking agents, sweeteners, and humectants[13]. Additionally, oligosaccharides exhibit prebiotic properties such as resistance to digestive enzymes and the ability to increase the population of probiotics, particularly *Bifidobacterium*, in murine and human gut. For the making of prebiotic or symbiotic food items, oligosaccharides, such as Xylooligosaccharides (XOS) are potential candidates due to these properties. XOS have been linked to potential health benefits beyond gut health including immune system modulation and improved mineral absorption due to their fermentation products[5].

The second-most plant based prevalent biopolymer, xylan, can be hydrolyzed to create XOS, that can be successfully recovered from lignocellulosic biomass[26]. Bamboo shoots, fruits, vegetables, milk, and honey are just some examples of the naturally occurring sources of xylooligosaccharides (XOs)[22]. Hardwoods, maize cobs, straws, bagasse, hulls, malt cakes and bran are typical raw materials used in the industrial production of XOS[9,11].

Alkali treatment is one strategy for XOS production where xylan-rich materials are subjected to alkaline hydrolysis. The process typically involves the use of sodium hydroxide (NaOH) or other alkali agents to disrupt the xylan structure, resulting in the release of xylooligosaccharides as well as xylose monomers[18]. However, this method can be less selective and may lead to the formation of undesirable byproducts. Enzymatic treatment, on the other side, relies on the application of specific enzymes capable of cleaving the glycosidic bonds within xylan molecules. Endo-1,4-β-xylanases and β-xylosidases are key enzymes involved in this process. Shorter xylan fragments are produced when endo-1,4- β xylanases cleave internal glycosidic connections in the xylan backbone. These fragments are then hydrolyzed further by β- xylosidases to produce xylooligosaccharides[37].

Enzymatic treatment offers better control over the size and composition of the resulting xylooligosaccharides, making it a preferred method for producing XOS with specific properties. XOS's significance in promoting gut health and potentially influencing broader physiological processes continues to be an active area of research and innovation.

Coconut husk is one of the lignocellulosic biomasses which can be explored for XOS production[25,26]. The diverse physiological effects of XOS and their potential impact on human health warrant further exploration and understanding, making them a subject of ongoing research and development[36].

Though various lignocellulosic biomasses have been experimented for XOS production, tender coconut husk was also studied for XOS production. Hence, the current work was done to evaluate the use of alkali treatment with steam to extract xylan from tender coconut husk followed by the

development of XOS, which can then be employed further in the food sectors as an effective prebiotic.

Material and Methods

Collection of Raw Materials and Characterization: Tender green coconut husk was collected from a local tender coconut shop. It was manually shred, and then dried at 55 °C in a hot air oven. The dried husk was then ground into a powder by using a lab-scale Wiley grinder. The powdered husk was sieved to get particle size of 1 mm. This powdered husk was then tested for moisture, ash, cellulose, lignin, and hemicellulose content. Cellulose and hemicellulose were analyzed using the Van Soest fiber analysis method[33] which separates plant cell components into less digestible (hemicellulose, cellulose, and lignin) and mostly digestible (starch and sugars) fractions using neutral and acid detergents as per AOAC[3] respectively.

Extraction of Xylan: The first step of xylan extraction was the alkali treatment. In this process, 0.5 g of powdered tender coconut husk was treated at a solid-to-liquid ratio of 1:10. The extraction of xylan was done by using varying concentrations of sodium hydroxide (0 – 20% w/v). Then the sample obtained from the alkali treatment was autoclaved (121°C and 15 psi pressure) for different time intervals (30, 45 and 60 minutes)[27]. All the experiments were done in triplicate. The acidification of the alkali-solubilised xylan to pH 5.0 was done by adding glacial acetic acid and then precipitated using ice-cold rectified spirit. The obtained xylan was precipitated by drying using hot air at 60°C till achieving uniform weight[28]. The dried xylan was weighed, and powdered with a lab-scale mixer, and stored at room temperature in sealed pouches.

True and relative xylan recovery were calculated [28] by the provided formula:

$$\text{True yield (\%)} = \frac{Dry\ weight\ of\ the\ estracted\ Xylan(g)}{weight\ of\ the\ Sample\ (g)}\ X\ 100$$

$$Relative\ Yield\ (\%) = \frac{True\ Yield}{Xylan\ present\ in\ original\ sample(g)}\ X\ 100$$

This produced xylan was later employed for FTIR analysis and for the extraction of XOS.

Production of XOS: The enzymatic method of hydrolysis of xylan was opted for XOS production over other extraction methods[11,17,27]. In this method, alkali-solubilized xylan (2%) was hydrolysed by crude xylanase enzyme obtained from Kaypees Biotech Pvt. Ltd., Mysore, India using sodium citrate buffer[2,11,28]. Samples were withdrawn periodically throughout the hydrolysis process[27]. The quantitative analysis of xylose and xylobiose was carried out using spectrophotometry as prescribed by Miller[21] and Mccleary and McGeough[20]. A mixture of 0.1 ml of the aliquot sample and 0.9 ml of distilled water was taken in a test tube. 3 ml of DNS reagent was added to this mixture. The test tubes were then placed in a boiling water bath for 15 minutes.

Subsequently, 1 milliliter of 40% Rochelle salt solution was added, thoroughly mixed and incubated at room temperature for approximately 15 minutes. The absorbance of the standard and samples was measured at 540 nm using evolution 201, UV-Visible spectrophotometer, against a reagent blank. To calibrate the results, standard graphs were plotted using xylose and xylobiose standards. To further analyze the best treatment based on xylobiose yield, a qualitative analysis was conducted. This involved drying the sample in a hot air oven (Leads, India) on trays measuring 37.7 × 15.4 cm at 55°C in a tray dryer until a constant weight was reached and the sample was subjected to FTIR analysis.

Process optimization of XOS production: To measure the influence of various parameters such as crude xylanase enzyme concentration (3% to 5%), pH (4 to 6), temperature (45 to 65 °C), and incubation time (6 to 24 hours) on the production of XOS from xylan, a statistical tool using Response surface methodology (RSM), Design Expert® version 13.0 software from Stat Ease Inc., Minneapolis, USA, was employed. A total of 30 experiments were conducted using a Face Centered Design (FCD) with four factors at three levels to explore the primary and higher-order effects of each factor and their interactions. The design had six center points with an alpha value of -1, 0, and +1.

The responses including xylose and xylobiose, and the variables such as temperature, enzyme concentration, time, and pH, were mathematically modeled using a quadratic equation. Each experiment was performed in triplicate, and the range and levels of these independent variables are tabulated in table 1. The experimental data from XOS production were subjected to Analysis of Variance (ANOVA) and Regression analysis. Polynomial equations of the second order were established based on the Analysis of Variance, and optimal parameters were determined using the optimization tools provided by Design Expert. To evaluate the quality of the model, standard deviation and R^2 values were calculated.

Table 1
Actual and Coded level of factors tested with RSM

Code	Factor	Unit	Coded levels		
			-1	0	1
A	Enzyme Concentration	mg/ml	3	4	5
B	Temperature	° Celsius	45	55	65
C	Time	Hour	6	15	24
D	pH		4	5	6

The mathematical model generated during the implementation of the Response surface methodology (RSM) was validated through checkpoint studies, with each case representing the average of triplicate experiments[26].

Results and Discussion

Dried Tender Coconut Husk's composition: The obtained results from the FTIR study of the dried tender coconut husk's content (Figure 1) revealed the percentage of various components in the material's dry weight. From the results, it was observed that lignin is the predominant component in the green tender coconut husk, constituting approximately 38.64 ± 0.15% of its dry weight followed by cellulose which makes up around 36.07 ± 1.15% of the dry weight of coconut husk. Hemicelluloses account for approximately 24.01±0.10% of the dry weight. Ash content in coconut husk is relatively low, at approximately 2.861±0.13% of the dry weight. In summary, the composition analysis of dried brown coconut husk reveals that it is primarily composed of lignin and cellulose, indicating its strength and durability.

Analysis of the Tender Coconut Husk by FTIR: The FTIR spectra results of tender coconut husk revealed various chemical components (Figure 1). A strong band at 3250.15 cm^{-1} indicates the presence of water[8]. Another at 2900.98 cm^{-1} signifies carbohydrates, and at 1675.98 cm^{-1}, acetyl groups may be present[16,23]. Bands at 1519.88 cm^{-1} and 1260.15 cm^{-1} are characteristic of lignin, with the latter representing aryl-alkyl ethers[8]. Additionally, 1046.78 cm^{-1} suggests the presence of cellulose. Some bands at 1675.98 cm^{-1} and 1519.88 cm^{-1} could relate to amino acids like asparagine, valine, phenylalanine, or tyrosine[30]. The band at 1260.15 cm^{-1} corresponds to serine. From these spectral characteristics, tender coconut husk's chemical composition and potential uses had been observed.

Yield of Xylan: In the present study, sodium hydroxide (NaOH) was the preferred method for extracting xylan from tender coconut husk, a type of lingo cellulosic biomass[4]. Tables 2 and 3 clearly illustrate that increasing the concentration of NaOH from 0% to 20% and extending the treatment time from 30 to 60 minutes led to a gradual and significant rise in both true and relative xylan yields. The only exception to this pattern was present in the control group (0% NaOH), where increasing the treatment time did not notably impact the true yield. The highest true yield, approximately 21% with a margin of error of ± 0.408%, was achieved using a 20% NaOH concentration and a 60-minute treatment. Conversely, the lowest yield, around 2% with a margin of error of ± 0.1%, was observed in the control group (0% NaOH) after a 30-minute treatment (Table 2). These findings underscore the effectiveness of NaOH in extraction of xylan from tender coconut husk and emphasize the significance of NaOH concentration and treatment time in optimizing the extraction process.

Treating lignocellulosic materials with alkali can cause them to swell, leading to increased internal surface area, reduced polymerization, decreased crystallinity, and the partition of bonds between carbohydrates and lignin. This degradation of lignin facilitates the easier extraction of xylan from lignocellulosic substrates[7]. The gradual raise in NaOH concentration resulted in a significant increase in the actual yield of xylan, suggesting that some hemicellulose is loosely attached to the matrix of the cell wall, while the maximum amount xylan is strongly embedded in the cell walls and requires higher alkali concentrations for extraction[14].

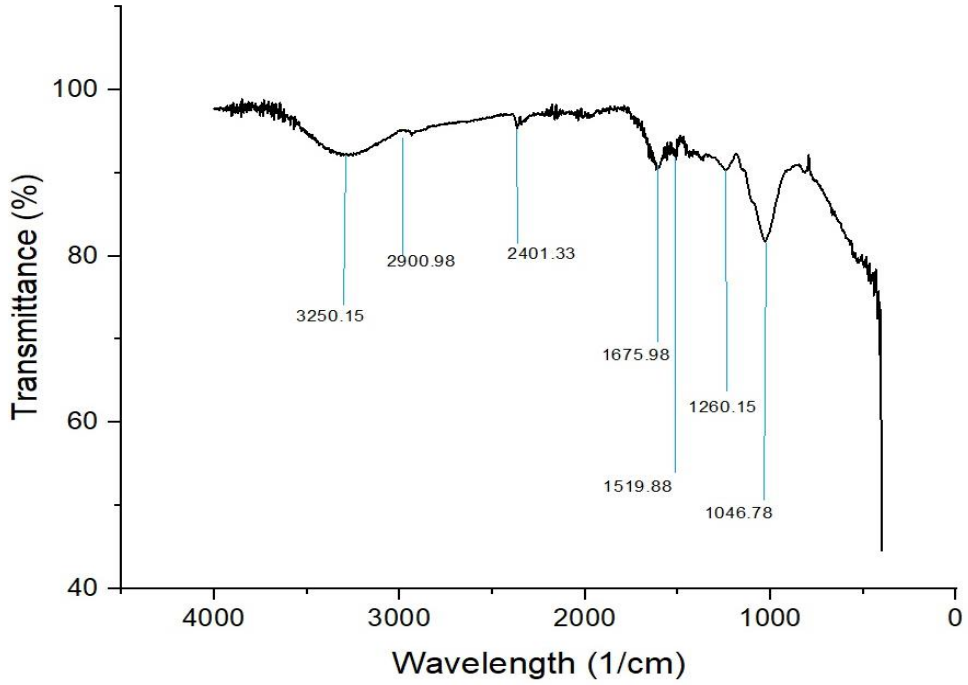

Fig. 1: FTIR spectra of tender coconut husk powder

Table 2
Effect of treatment time and NaOH concentration on the true yield of xylan from tender coconut husk

NaOH concentration (%)	Treatment time		
	30 min	45 min	60 min
	True yield (%)		
0	2±0.1	2±0.78	2±0.821
4	7±0.91	9±0.11	8±0.22
8	9±0.17	12±0.88	13±0.97
12	12±0.24	16±0.17	16±0.79
16	15±0.78	17±0.12	19±0.45
20	18±0.18	19±0.33	21±0.408

Table 3
Effect of treatment time and NaOH concentration on the relative yield of xylan from tender coconut husk

NaOH Concentration (%)	Treatment time		
	3o min	45 min	60 min
	Relative yield (%)		
0	16±0.962	17±0.46	18±0.34
4	19±0.966	25±0.87	26±0.5
8	35±0.892	28±0.956	34±0.3
12	49±0.325	35±0.984	58±0.28
16	53±0.484	45±0.12	63±0.193
20	63±0.7008	64±0.44	82±0.816

Therefore, NaOH proved effective in the separation of xylan from the complex structure of tender coconut husk, especially at larger concentrations. In terms of relative recovery, almost 82% of the xylan was recovered from the tender coconut husk powder after treatment with 20% NaOH and steam for 60 minutes (Table 3). Other lingo cellulosic materials such natural grass (98% xylan)[28], pigeon pea stem (96% xylan)[29], corn cobs (83% xylan)[27] and sugarcane bagasse (85% xylan)[14] have all shown comparable results.

Analysis of Xylan by FTIR: The spectra from FTIR in figure 2 illustrate the properties of obtained xylan from tender coconut husk using a combination of NaOH treatment and steam. Several distinct peaks are evident including prominent bands at 3419.90, 2926.11, 1645.33, 1514.17, and 1423.51, among others. The band at 3410.16 cm^{-1} corresponds to the elongation of OH groups bonded by hydrogen within the extracted xylan. Likewise, the existence of the 2904.55 cm^{-1} band signifies C–H stretching in the xylan, a characteristic that has been documented in previous study on xylan extracted from pigeon pea stalk[29] and sugarcane bagasse[24].

Remarkably, the non-appearance of the 1660.91 cm^{-1} band in the extracted xylan using alkali, which was evident in the untreated tender husk (as depicted in figure 1), indicates the effective removal of O-acetyl groups within the hemicellulose chain through alkali treatment [23]. This specific band at 1660.91 cm^{-1} may be associated with the pliant mode of absorbed water, given that xylans are known for their strong affinity for water[15,24,29]. The band at 1523.76 cm^{-1} signifies the lingering presence of bound lignin, representing that the process of extraction did not entirely eliminate the binded lignin from the husk xylan. This finding is consistent with prior research on xylan extraction from pigeon pea stalk[29]. The band at 1425.116 cm^{-1} may be attributed to the bending vibrations involving C–H, C–O, or C–OH within the xylan structure[29].

The distinctive cellulose band at 1046.78 cm^{-1}, seen in the tender coconut husk (Figure 1), is conspicuously not present in the extracted xylan, signifying the successful separation of hemicellulose from the covalently linked hemicellulose-cellulose complex. Lastly, the robust signal at 1040.86 cm^{-1} corresponds to C–O, C–C stretching, or C–OH binding within xylan, while the stretch at 892.29 cm^{-1} indicates the C1 group frequency or ring frequency, which characterizes beta xylosidic linkages between sugar monomers. The band at 660.12 cm^{-1} may be linked to C–C–H stretching, and the one at 526.11 cm^{-1} to the bending of C–OC[14,19].

Optimization and Production of XOS: To analyze the effect of process variables on the XOS production with respect to xylose and xylobiose, 30 experiments were conducted according to the central composite design (CCD) (Table 4). The experiments were conducted to decide the effect of enzyme concentration, temperature, time and pH on XOS production. From the experiments, it was observed that all the process variables had Hadlinear effect on xylose and xylobiose except for pH. The value of the correlation coefficient (R^2) for xylose was found to be 0.9203 (Table 5) and xylobiose to be 0.9170 (Table 6) indicating a good fit. Model Summary and statistics are given in tables 5 and 6.

Because of high R-Squared, a quadratic model with an adjusted R-Squared value of 0.8459 for xylose and 0.8396

for xylobiose predicted the R-Squared value of 0.6798 for xylose and 0.6710 for xylobiose respectively opted for further analysis.

The distribution of experimental and predicted values of response variable of concentration of xylose and xylobiose is shown in figures 3 and 4 implying the fitness of proposed RSM model.

Table 4
CCD design matrix for the optimization of process variables and the Responses for Xylose and Xylobiose during XOS production

Run	Process Variables				Responses	
	Enzyme concentration	pH	Temperature (ºC)	Time (hrs)	Xylose(mg/ml)	Xylobiose(mg/ml)
1	3	65	6	6	2.33	1.73
2	4	55	6	5	2.3	1.7
3	3	55	15	5	2.37	1.73
4	4	55	15	4	2.14	1.54
5	4	55	15	5	2.59	1.99
6	3	45	24	6	2.11	1.51
7	3	65	24	4	1.88	1.28
8	3	65	24	6	1.95	1.35
9	4	55	15	5	2.59	1.99
10	5	65	24	6	2.61	2.01
11	5	45	6	6	2.01	1.41
12	5	45	24	4	2.41	1.81
13	3	45	6	4	1.77	1.17
14	4	55	24	5	2.54	1.94
15	5	45	24	6	2.34	1.74
16	4	45	15	5	2.34	1.74
17	4	55	15	6	2.43	1.83
18	4	55	15	5	2.48	1.88
19	3	45	24	4	2.05	1.45
20	5	45	6	4	2.01	1.41
21	4	55	15	5	2.63	2
22	3	65	6	4	1.91	1.31
23	4	65	15	5	2.31	1.71
24	3	45	6	6	1.87	1.27
25	5	65	24	4	2.58	1.98
26	5	65	6	6	2.85	2.21
27	4	55	15	5	2.64	1.99
28	5	55	15	5	2.49	1.88
29	4	55	15	5	2.63	1.99
30	5	65	6	4	2.55	1.95

Table 5
Model Summary statistics for Xylose

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.2172	0.4838	0.4012	0.2227	1.77	
2FI	0.1966	0.6785	0.5093	0.0105	2.26	
Quadratic	**0.1102**	**0.9203**	**0.8459**	**0.6798**	**0.7312**	**Suggested**
Cubic	0.1042	0.9667	0.8622	-2.5444	8.09	Aliased

Table 6
Model Summary statistics for Xylobiose

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.2203	0.4680	0.3829	0.2037	1.82	
2FI	0.2023	0.6590	0.4795	-0.0491	2.39	
Quadratic	**0.1123**	**0.9170**	**0.8396**	**0.6710**	**0.7504**	**Suggested**
Cubic	0.1054	0.9659	0.8587	-2.5899	8.19	Aliased

The Analysis of Variance (ANOVA) of RSM model is given in table 7 for xylose and table 8 for xylobiose respectively. ANOVA gives the significance of model and testing variables. In this model, the P_{model}value (Fisher's F-test) of the model is very much less than the F value (Pmodel = 0.0001). This indicates that the model is significant. Our regression equations accounted for 92.03% ($p < 0.001$) of the total variation in xylose and 91.70% ($p < 0.001$) of the total variation in xylobiose respectively. The significance of enzyme concentration is more dominant than all other parameters in XOS production.

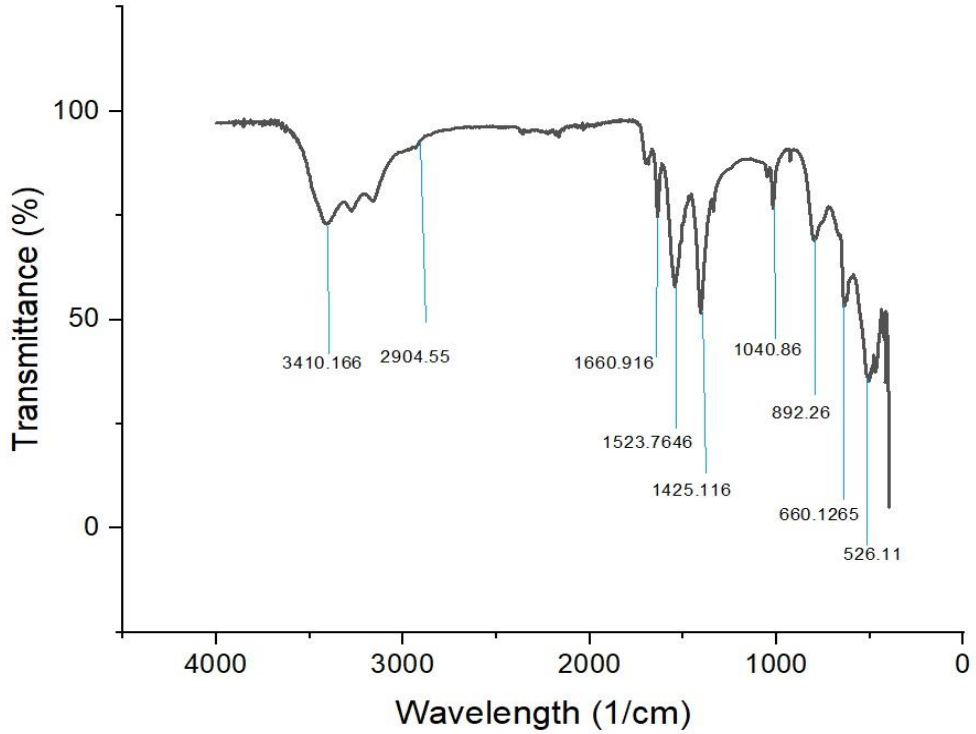

Figure 2: FTIR spectra of xylan obtained from tender coconut husk through 20% NaOH treatment coupled with steam treatment

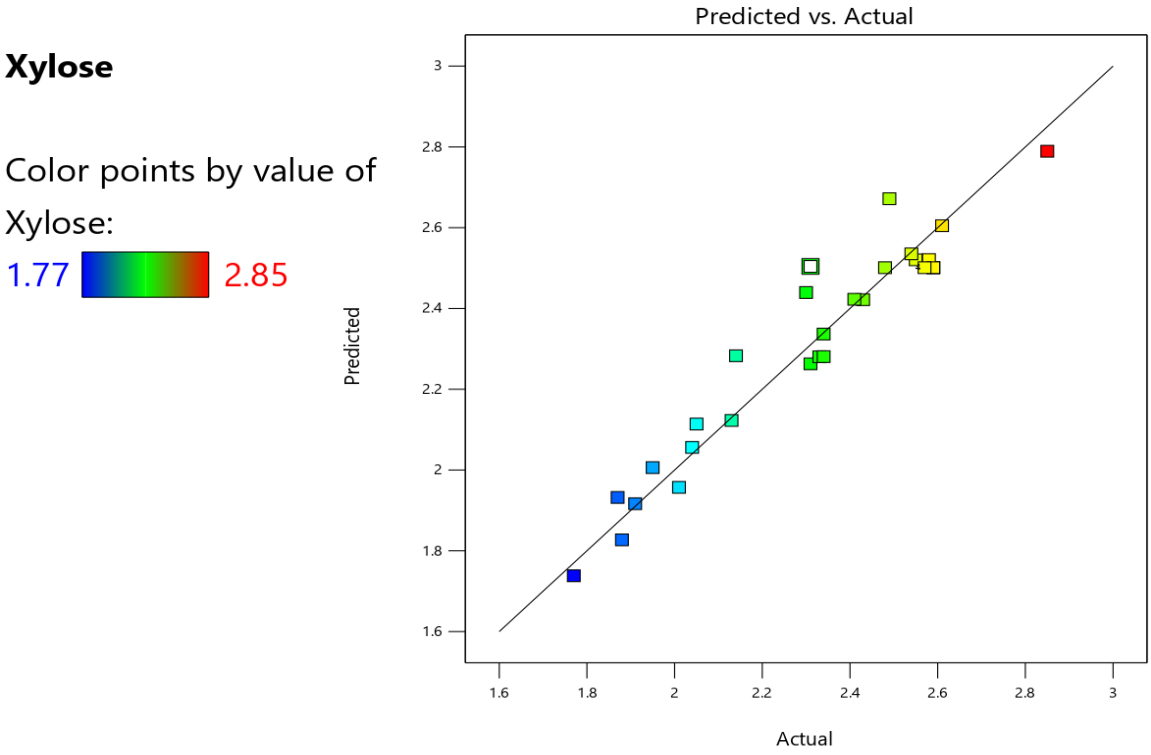

Figure 3: Correlation between experimental and predicted Xylose yield

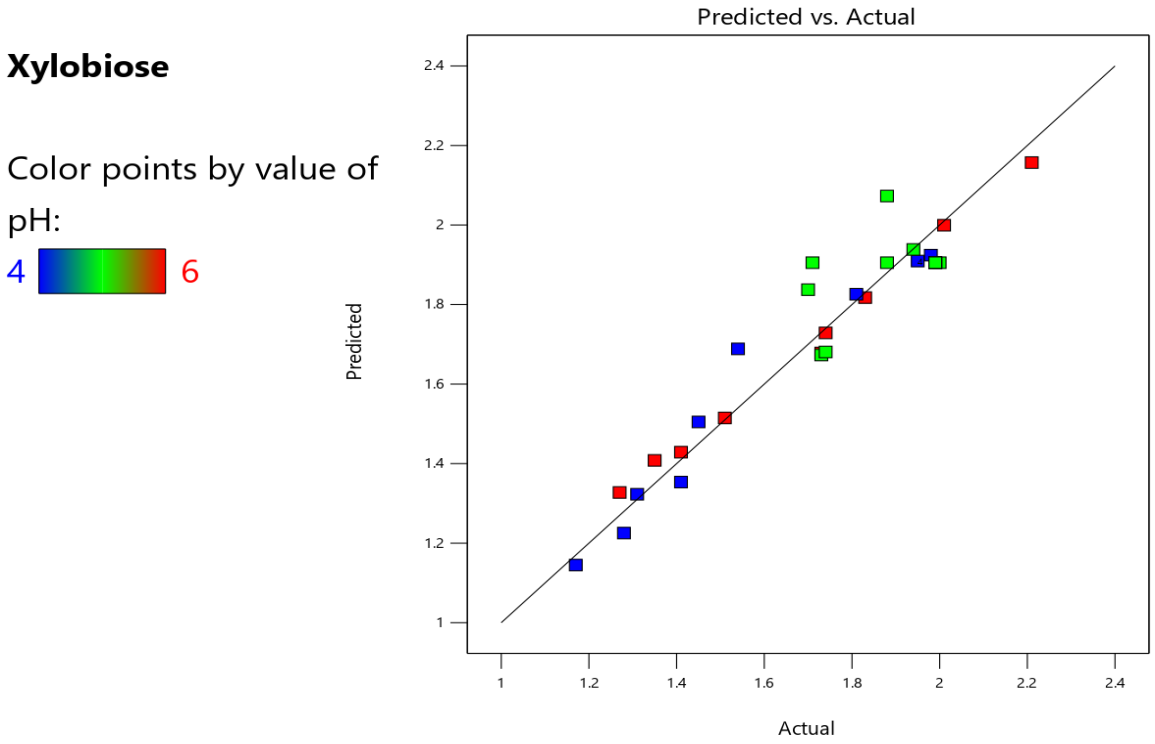

Figure 4: Correlation between experimental and predicted Xylobiose yield

Table 7
ANOVA for yield of Xylose during XOS production

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.10	14	0.1501	12.37	< 0.0001	significant
A-Enzyme Concentration	0.7524	1	0.7524	61.99	< 0.0001	
B-Temperature	0.2245	1	0.2245	18.49	0.0006	
C-Time	0.0411	1	0.0411	3.39	0.0856	
D-pH	0.0868	1	0.0868	7.15	0.0173	
AB	0.1482	1	0.1482	12.21	0.0033	
AC	0.0081	1	0.0081	0.6674	0.4268	
AD	0.0090	1	0.0090	0.7436	0.4021	
BC	0.2162	1	0.2162	17.82	0.0007	
BD	0.0289	1	0.0289	2.38	0.1436	
CD	0.0342	1	0.0342	2.82	0.1138	
A^2	0.0029	1	0.0029	0.2409	0.6306	
B^2	0.0306	1	0.0306	2.52	0.1334	
C^2	0.0005	1	0.0005	0.0395	0.8452	
D^2	0.0572	1	0.0572	4.71	0.0464	
Residual	0.1821	15	0.0121			
Lack of Fit	0.1724	10	0.0172	8.90	0.0131	significant
Pure Error	0.0097	5	0.0019			
Cor Total	2.28	29				

Contour plots were constructed to find out the optimum level of each factor which maximizes the yield of xylose and xylobiose. These plots estimate the yield of xylan and xylobiose on two factors at a time, while other factors are fixed at its central level. Figures 5, 6, and 7 demonstrate that an increase in enzyme concentration leads to a gradual and significant rise in xylose yield. This can be attributed to the degradation of xylooligosaccharides (XOS) into xylose monomers[2,27,29]. Concerning temperature, elevating the temperature of incubation results in a gradual and significant increase in xylose yield because of enhanced breakdown of XOS into monomers (Figure 5). On the other hand, increasing the time of incubation initially leads to a steady increase in the yield of xylose followed by a slight, non-

significant decrease (Figure 6). This could be the reason for the hydrolysis of XOS and xylan into xylose monomers with prolonged incubation time[27]. Conversely, with respect to increase in pH, there is an initial non-significant increase followed by a slight decrease in xylose yield from tender coconut husk xylan (Figure 7). This pattern may be due to higher pH favouring the production of xylobiose and xylotriose oligomers over monomeric xylose[2,27,29].

Concerning xylobiose, an increase in enzyme concentration resulted in a significant yield increase (Figures 8, 9, 10), as higher enzyme concentrations expedite the hydrolysis of xylan into xylooligosaccharides (XOS) and xylose[27,29]. In terms of temperature, the initial increase in the yield of xylobiose was followed by a gradual decrease (Figure 8). This decline resulted in further hydrolysisation of XOS into its monomer, xylose[14,27]. There was a gradual increase in xylobiose production with respect to the increase in incubation time (Figure 9), and it is because of the ongoing hydrolysis of xylan into XOS. Similarly, a slight increase in xylobiose yield was observed with increasing pH (Figure 10), possibly because higher pH encourages oligomer production over monomer formation as previously explained[27,29].

Table 8
ANOVA for yield of Xylobiose during XOS production

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.09	14	0.1494	11.84	< 0.0001	Significant
A-Enzyme Concentration	0.7200	1	0.7200	57.07	< 0.0001	
B-Temperature	0.2267	1	0.2267	17.97	0.0007	
C-Time	0.0460	1	0.0460	3.65	0.0755	
D-pH	0.0748	1	0.0748	5.93	0.0279	
AB	0.1425	1	0.1425	11.30	0.0043	
AC	0.0127	1	0.0127	1.00	0.3324	
AD	0.0116	1	0.0116	0.9160	0.3537	
BC	0.2093	1	0.2093	16.59	0.0010	
BD	0.0298	1	0.0298	2.36	0.1454	
CD	0.0298	1	0.0298	2.36	0.1454	
A^2	0.0027	1	0.0027	0.2152	0.6494	
B^2	0.0327	1	0.0327	2.59	0.1282	
C^2	0.0008	1	0.0008	0.0619	0.8068	
D^2	0.0602	1	0.0602	4.77	0.0453	
Residual	0.1892	15	0.0126			
Lack of Fit	0.1787	10	0.0179	8.48	0.0146	significant
Pure Error	0.0105	5	0.0021			
Cor Total	2.28	29				

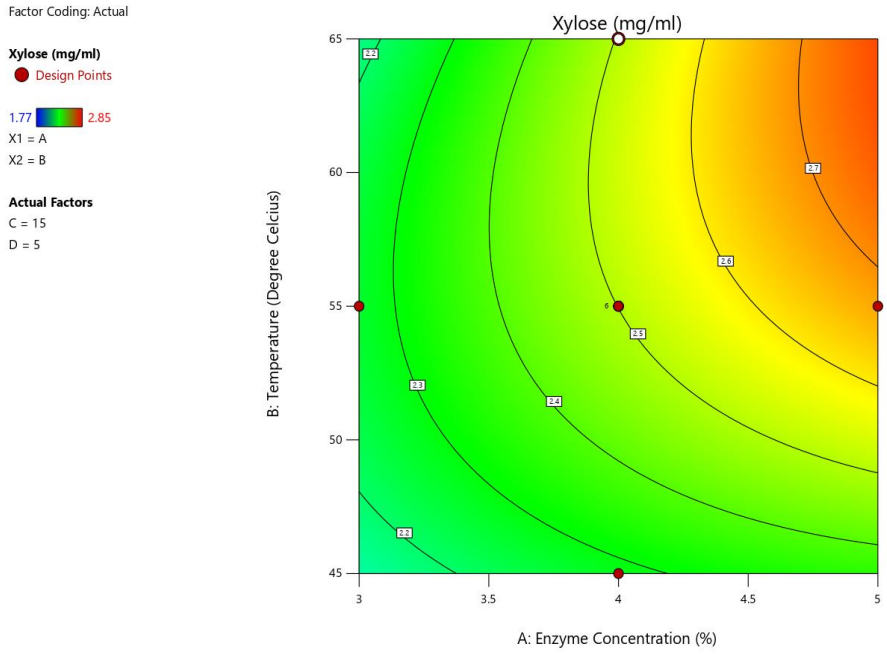

Figure 5: Contour plot for the correlation of Enzyme concentration and Temperature on Xylose Yield

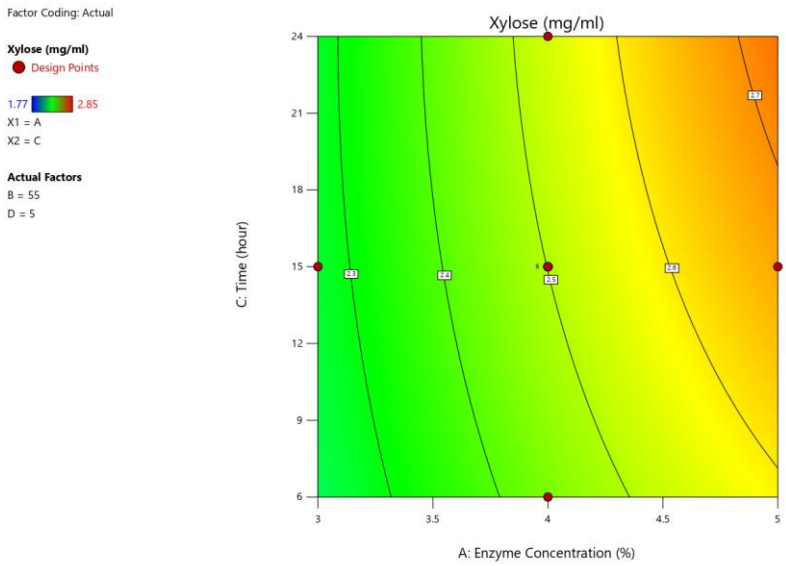

Figure 6: Contour plot for the correlation of Enzyme concentration and Time on Xylose Yield

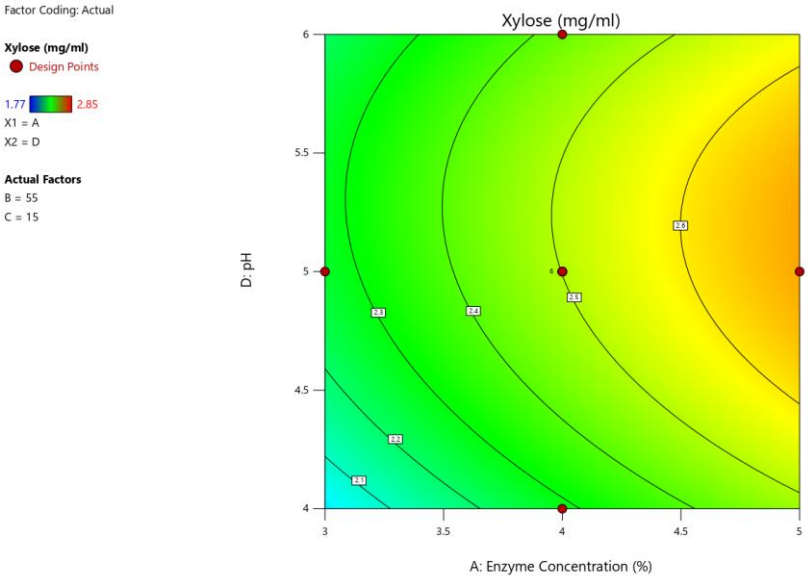

Figure 7: Contour plot for the correlation of Enzyme concentration and pH on Xylose Yield

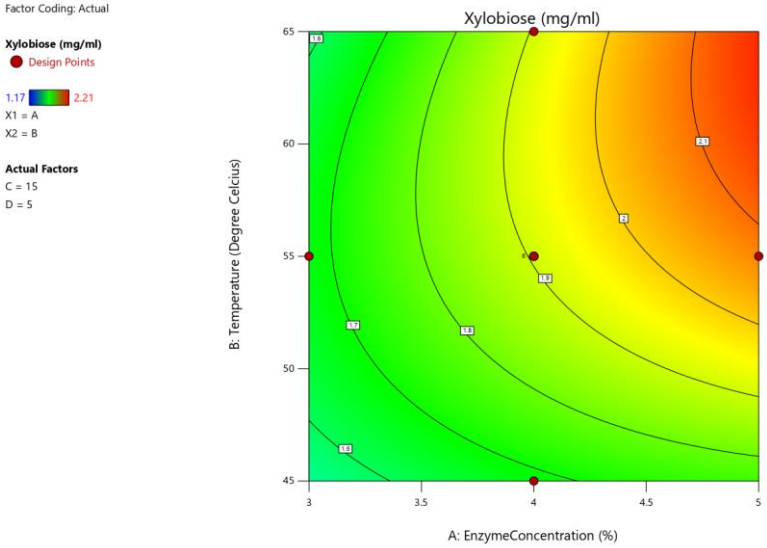

Figure 8: Contour plot for the correlation of Enzyme concentration and Temperature on Xylobiose Yield

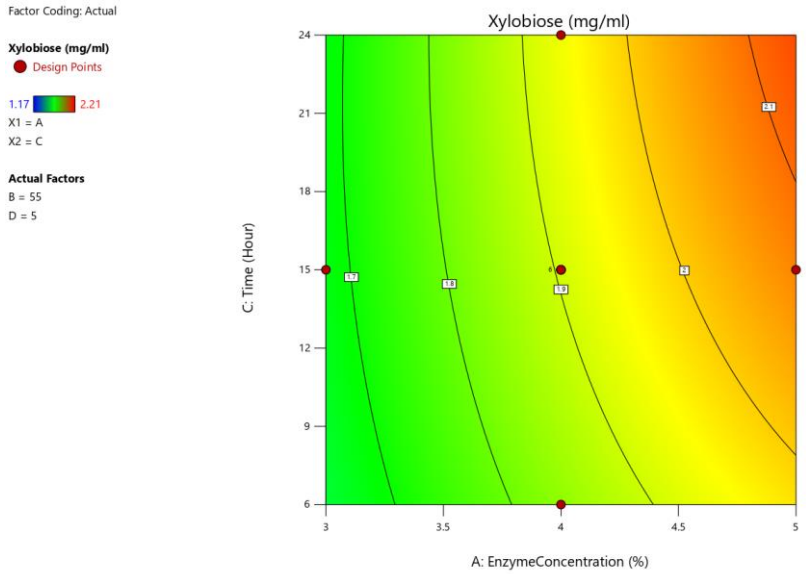

Figure 9: Contour plot for the correlation of Enzyme concentration and Time on Xylobiose Yield

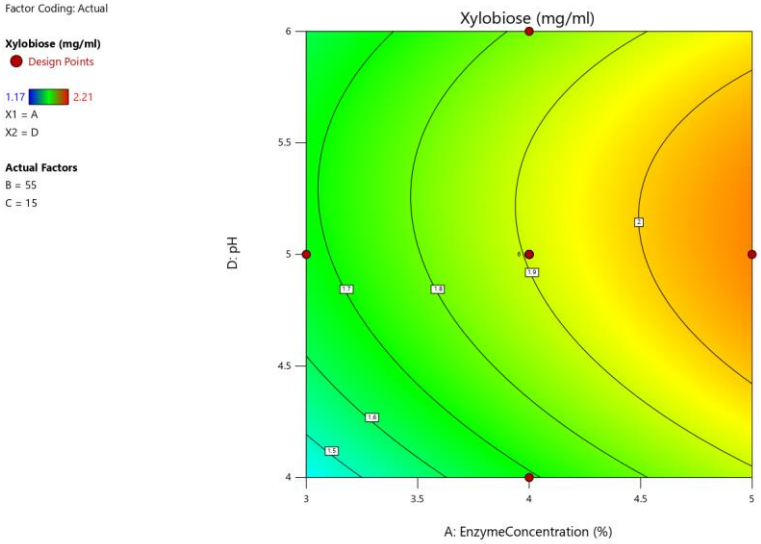

Figure 10: Contour plot for the correlation of Enzyme concentration and pH on Xylobiose Yield

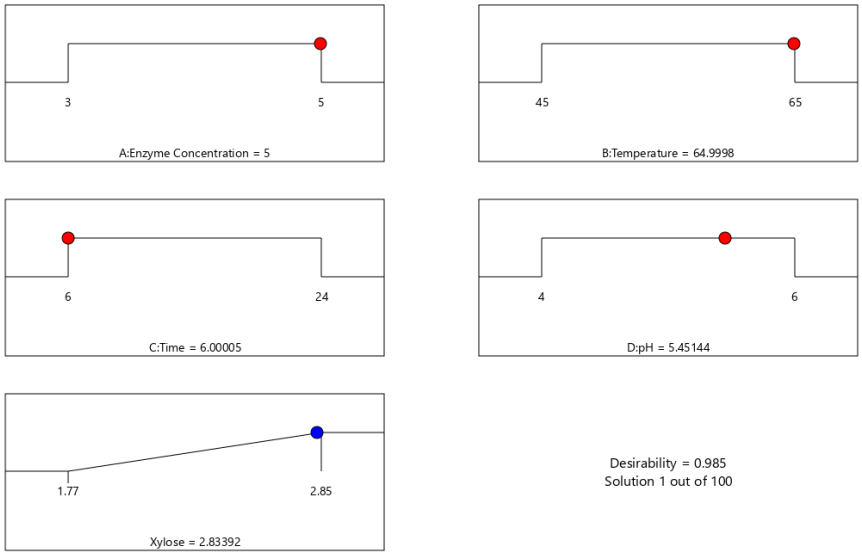

Figure 11: Numerical Optimization for Xylose Production using RSM

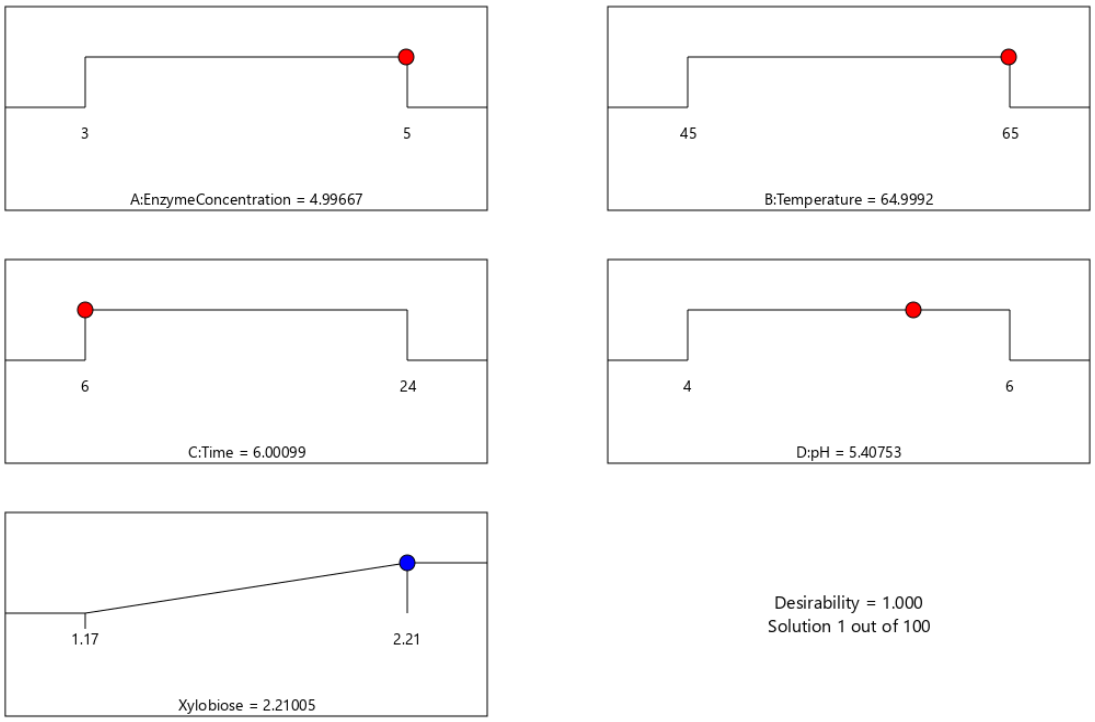

Figure 12: Numerical Optimization for Xylobiose Production using RSM

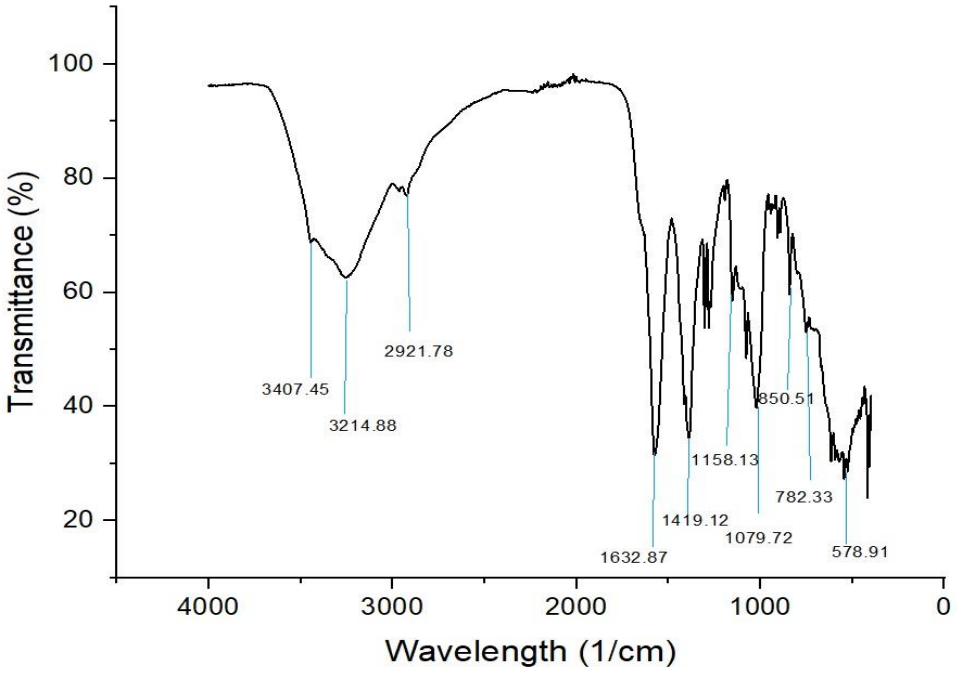

Figure 13: FTIR spectra of XOS obtained from enzymatic hydrolysis of tender coconut husk xylan

To optimize production of xylobiose and xylose from xylan (Figure 10, 11), we employed a numerical optimization tool of RSM. The optimum conditions for XOS production were found to be crude xylanase 5.0% enzyme concentration, pH 5.4, temperature 65°C, and 6 hours of incubation time with desirability score of .0.985 for xylose and 1 for xylobiose respectively. These conditions yielded 2.834mg/ml of xylose and 2.21mg/ml of xylobiose. The yields of xylose and xylobiose obtained in the present study were comparable to those documented in the literature for other lignocellulosic substances such as sugarcane bagasse, pigeon pea stalk, corn cobs, and natural grass under various optimized conditions[27-29].

Analysis of Xylooligosaccharides by FTIR: The FTIR spectra of the XOS mixture, known as XOS mix, displayed significant peaks at 3407.45, 2921.78, 1632.87, 1419.12, 1079.72, and 850.51 cm^{-1} (Figure 13). This spectral pattern

closely resembles the FTIR analysis of XOS mix obtained from brown coconut husk[26]. The absorption peak at 3407.45 cm^{-1} corresponds to O–H bond stretching. Additionally, the peaks at 2921.78 and 1632.87 cm^{-1} are indicative of C–H stretching in carbohydrate molecules. The peak at 1079.72 cm^{-1} represents the stretching of C–O, C–C–C, and C–OH bonds, which are the characteristics of hemicelluloses[14,19].

This predicts the existence of xylan groups within the XOS mix in alignment with previous reports[19]. As mentioned earlier, the band at 894 cm^{-1} signifies the presence of glycosidic bonds between sugar molecules [6]. Correspondingly, the band at 850.51 cm^{-1} in the current study (Figure 13) may be associated with these glycosidic bonds[6,26].

Conclusion

This research underscores the potential of tender coconut husk, often overlooked as a byproduct, as a valuable source for extracting xylan and xylooligosaccharides which are short-chain carbohydrates derived from xylan and have gained consideration for their health benefits and industrial applications. The study successfully demonstrated an effective method for extraction of xylan from tender coconut husk using alkali and steam treatment followed by enzymatic hydrolysis to produce XOS.

From the chemical analysis, the presence of hemicelluloses, cellulose, lignin, and ash in the husk, had been confirmed affirming its suitability for XOS extraction. Using response surface methodology, optimal conditions were identified, with respect to concentration of enzyme and temperature significantly affecting xylose yield, and enzyme concentration, temperature, time, and pH influencing xylobiose yield. FTIR analysis provided further evidence of hemicelluloses in the tender coconut husk and the presence of xylan in the extracted material.

Under the optimized conditions involving a combination of 20% NaOH treatment coupled with steam, a crude xylanase enzyme concentration of 5.0%, a pH of 5.4, a temperature of 65°C, and a 6-hour incubation time, an impressive desirability score of 1 were achieved. This led to the production of 2.834 mg/ml of xylose and 2.21 mg/ml of xylobiose. In conclusion, this study not only highlights the potential of coconut husk as a resource for xylan and XOS extraction but also emphasizes the promising health benefits of XOS, particularly as prebiotics that can contribute to improved gut health. These findings open up new avenues for the sustainable utilization of agricultural residues and the development of functional food ingredients with significant health implications.

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