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Optimization of Xylitol Production from Tobacco Stem Using Ultrasound-Assisted Acid Hydrolysis Coupled with Fermentation

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Abstract

Tobacco (Nicotiana tabacum L.), as one of the main commodities in East Java Province, where only the leaves are utilized, while the stems become agricultural waste and are often burned, contributes to air pollution. Nonetheless, these tobacco stems possess potential for high-value chemical products, such as xylitol. This study investigated the optimum operating conditions for producing xylitol from tobacco stem waste. The process employed Ultrasound-Assisted Acid Hydrolysis followed by fermentation using Candida tropicalis. Hydrolysis was conducted in an ultrasonic bath that operates at 40 kHz and 50 watts, using 0.1 N H_2SO_4 as the solvent. The resulting hydrolysate was then fermented by adding Candida tropicalis (20 mL per 100 mL substrate) and incubating for 48 hours, 30 °C, 120 rpm shaker. Optimization was conducted utilizing Response Surface Methodology method to assess the interactions between operating variables (temperature, time, and volume of solvent during hydrolysis) and xylitol yield. The study investigated the optimum operating conditions to produce xylitol were 30 °C, 50 minutes, and 150 mL of solvent, yielding xylitol at a concentration of 5.6 g/L.

Keywords: *Xylitol, Tobacco, Ultrasound-Assisted Acid Hydrolysis, Fermentation, Optimization.*

Abstrak

Tembakau (*Nicotiana tabacum* L.) sebagai komoditas perkebunan andalan di Provinsi Jawa Timur yang selama ini pemanfaatannya hanya pada daun, menimbulkan masalah polusi udara karena limbah batang sering kali dibuang dan dibakar. Sementara itu, batang tembakau berpotensi besar untuk dimanfaatkan sebagai produk bernilai tinggi, termasuk xylitol. Penelitian ini bertujuan untuk memperoleh kondisi operasi optimum dalam produksi xylitol dari limbah batang tembakau. Proses diawali dengan mendapatkan hidrolisat melalui Ultrasound-Assisted Acid Hydrolysis, lalu dilanjutkan dengan proses fermentasi menggunakan mikroorganisme *Candida tropicalis*. Hidrolisis dilakukan dalam bak ultrasonik pada kondisi operasi 40 kHz dan 50 watt, dengan pelarut H_2SO_4 0,1 N. Hidrolisat yang diperoleh kemudian difermentasi dengan penambahan *Candida tropicalis* sebanyak 20 mL per 100 mL substrat, dan diinkubasi 48 jam, suhu 30 °C, di dalam shaker berkecepatan 120 rpm. Optimasi dilakukan dengan metode *Response Surface Methodology* untuk mempelajari pengaruh hubungan antar variable proses meliputi suhu, waktu, dan volume pelarut terhadap hasil xylitol. Dari hasil penelitian, diperoleh kondisi optimum pada, suhu 30 °C, waktu 50 menit, dan volume pelarut 150 mL yang mampu menghasilkan xylitol dengan konsentrasi 5,6 g/L.

Kata Kunci: Xylitol, Tembakau, Hidrolisis Asam Terbantukan Ultrasonik, Fermentasi, Optimasi.

1. INTRODUCTION

Tobacco is one of the leading commodities in Indonesia's agricultural sector, with its production center located in East Java, where an average of 117.930 tons was produced between 2019 and 2023 (Ministry of Agriculture, 2024). Tobacco plants generally only use the leaves, while tobacco stems will be considered agricultural waste and then burned (Amirudin et al., 2020), contributing to air pollution. Tobacco stems contain 22,44% hemicellulose (Slamet et al., 2022). This content shows the great potential of tobacco stem waste to produce high-value products, namely xylitol (Pramasari et al., 2023), thus helping to reduce environmental impacts.

Xylitol ($C_5H_{12}O_5$) is a natural sweetener commonly utilized as a substitute for sucrose because it offers a comparable sweetness level, namely 0.8 - 1.0, but xylitol has a caloric value of 2.4 calories/gram, while sucrose has 4 calories/gram (Purnawan et al., 2021). Several health benefits have been associated with the consumption of xylitol, such as preventing tooth decay by inhibiting bacterial growth and reducing constipation, diabetes, obesity, and other health conditions (Gasmi Benahmed et al., 2020). The growing demand is supported by its various benefits, which have led to its wide utilization in the food and healthcare industries (Kaur et al., 2023).

In this study, the process of making xylitol from tobacco stem waste raw materials uses hydrolysis and fermentation methods. Xylitol can be synthesized through chemical and biotechnological pathways, meaning fundamental stages include obtaining xylose by acid hydrolysis and xylose conversion into xylitol via catalytic hydrogenation (Delgado Arcaño et al., 2020) or fermentation (Umai et al., 2022). Ultrasound-Assisted Acid Hydrolysis (UAAH) was used in this study, which is known to be simpler, energy-saving, and time-saving when compared to conventional hydrolysis (Flores et al., 2021). UAAH is a process that combines ultrasound-assisted extraction and acid hydrolysis simultaneously. Ultrasound-assisted extraction utilizes high-frequency sound waves (15 kHz to 500 MHz) to create and collapse cavitation bubbles, inducing chemical and physical effects that enhance solvent penetration and disrupt cell walls (Najjoum et al., 2025). While acid hydrolysis is a widely applied technique for extracting hemicellulose, where acids like sulfuric acid (H_2SO_4) or hydrochloric acid (HCl) are used to break the glycosidic linkages in hemicellulose, converting it into individual monosaccharide units (Gautam et al., 2025). Fermentation or biological method is favored because it has lower cost and energy than the chemical method, and the bioconversion-derived xylitol is safer for food use and more environmentally friendly, avoiding metal catalyst residues (Umai et al., 2022). In fermentation, xylitol is synthesized through processes involving microorganisms or enzymatic pathways (Subroto, 2020).

The fermentation process in this research was assisted by microorganisms, namely *Candida tropicalis*. *Candida tropicalis* was inoculated into the substrate and subsequently introduced into the fermenter (Slamet et al., 2022). In general, microorganisms from the genus Candida are the most widespread microorganisms for producing xylitol (Subroto, 2020). This study has compared several types of Candida, including *Candida tropicalis*, *Candida guilliermondii*, and *Pichia stipitis*; therefore, the selection of *Candida tropicalis* as a microorganism is due to the results of maximizing xylitol from xylose, reaching 82.69% compared to other Candida microorganisms, so *Candida tropicalis* is one of the solutions in the xylitol fermentation process. Other researchers have also reported that *Candida tropicalis* has been successfully employed for the bioconversion of xylitol. The previous research achieved a yield of 55,8% from corn cob xylose (Fairus et al., 2013), 31.1% from sugar cane (Cardoso & Forte, 2021), 97.1% from a xylose mother liquor (L. Zhang et al., 2021), and 5.88% from areca nut skin (Vardhan et al., 2023), likewise this study addressed *Candida tropicalis* fermentation to produce xylitol from tobacco stem waste that has not been investigated.

Although xylitol production from lignocellulosic biomass has been widely studied, research rarely explores tobacco stem waste. At the same time, Ultrasound-Assisted Acid Hydrolysis (UAAH) represents a potential method, as it facilitates more effective cell wall breakdown and enhances solvent access, while *Candida tropicalis* is known for its ability to convert xylose into xylitol effectively, yet its use for tobacco stem biomass is still very limited. This study aims to fill that gap by combining UAAH and microbial fermentation in an approach to produce xylitol from tobacco stem waste under optimized conditions has not been thoroughly explored. By using Response Surface Methodology (RSM) to fine-tune the key process variables, this research promotes the utilization of an overlooked biomass source and an environmentally friendly pathway for xylitol production.

This study applied Ultrasound-Assisted Acid Hydrolysis to extract xylose from tobacco stem and utilized Candida tropicalis to convert xylose into xylitol. The operating conditions used were solvent volume, temperature, and time. The optimization using *Response Surface Methodology* was performed to obtain the optimum operating condition that contributed to xylitol yield.

2. METODHOLOGY

2.1 Pretreatment

The research methodology was presented in Figure 1. The experimental procedure was structured into four main stages: pretreatment, ultrasound-assisted acid hydrolysis, fermentation, and purification.

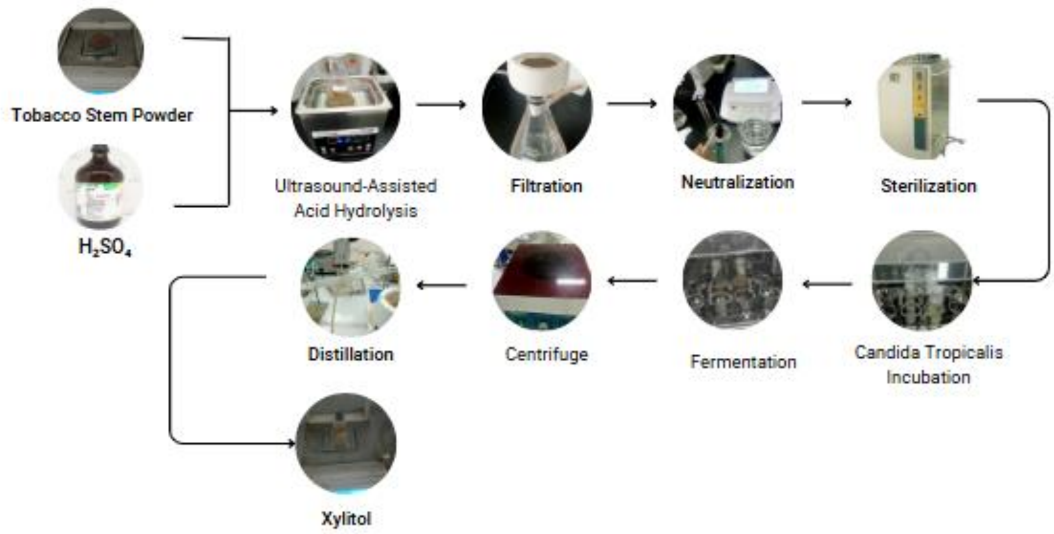


Figure 1. Graphical summary of the methodology

Tobacco stems were dried with the help of sunlight until dry and the entire stem was brownish. The dried tobacco stems were then reduced in size. The tobacco stems that have been reduced in size were then subjected to an oven, dried at 60°C for 60 minutes. After the drying process, the tobacco stems were ground and then sieved using a 100 mesh sieve.

2.2 Experimental design

Optimization of variable variations using Design Expert software with the Response Methodology Surface (RSM). The selection of Design Expert software was carried out because the software provides complete features for statistical analysis of experiments that facilitate the relationship between process variables and the RSM method of the Box Behnke Design (BBD) type was chosen (Diantoro et al., 2022), to design a research model that describes the interaction between temperature, time, and solvent volume on yield of xylitol from tobacco stem waste.

The independent variables in this study include temperature, time, and the raw material to solvents ratio. The temperatures used were 30°C, 40°C, and 50°C. The times used were 40, 50, and 60 minutes. The raw material to solvent ratios used were 1:10, 1:20, and 1:30. The variables presented in Table 1 were used to obtained the amount of experimental data presented in Table 2. Seventeen experiments (runs) were carried out following the BBD design for three factors at three levels.

Table 1. Operating condition limits

Parameter	Lower limit	Upper limit
Temperature (°C)	30	50
Time (minutes)	40	60
Solvent volume (mL)	50	150

2.3 Ultrasound-Assisted Acid Hydrolysis (UAAH)

Hydrolysis was carried out in a batch ultrasonic bath using Ultrasound-Assisted Acid Hydrolysis (UAAH) method, with operating parameter used being frequency of 40 kHz and power of 50 watts (Sasongko & Legahati, 2020). The mass of tobacco stem powder used was 5 grams. The illustration for the Ultrasound-Assisted Acid Hydrolysis (UAAH) method in a batch system is presented in Figure 1.

The acid solvent used during the hydrolysis process was H_2SO_4 0.1 N (Fairus et al., 2013). Then, the sample was processed using the Ultrasound-Assisted Acid Hydrolysis (UAAH) method in a batch process according to the variable variation presented in Table 1. After the hydrolysis process, the sample was left for 12 hours at room temperature. The hydrolysate was obtained using a vacuum pump. The hydrolysate obtained was adjusted with NaOH to a pH of 5.5. The resulting solution was then separated using a centrifuge at 600 rpm for 20 minutes. After the desired hydrolysate was obtained, fermentation will be carried out with *Candida tropicalis* microorganisms.

2.4 Fermentation

The inoculum was aseptically prepared by inoculating a full loop of agar medium to 20 mL of nutrient broth in a 100 mL Erlenmeyer flask, then incubated in an incubator shaker at 30°C, 150 rpm, and 24 hours (Ayu et al., 2021). The hydrolysate was sterilized using an autoclave at 121°C before being added to the fermentation medium, and then the hydrolysate was left to cool to room temperature (Fairus et al., 2013). The fermentation process was carried out following the method conducted by Fairus et al. The fermentation process was carried out in a 250 mL Erlenmeyer flask. *Candida tropicalis* inoculation was carried out as much as 20 mL/100 mL of substrate and put into the fermenter. The culture was incubated for 48 hours at 30°C using a shaker incubator at a speed of 120 rpm.

2.5 Purification

The purification process was carried out in a centrifuge and distillation. The fermented solution was flowed into a centrifuge at a speed of 600 rpm for 20 minutes. This was done to separate *Candida tropicalis* from the mixture so that a mixture of water and xylitol was obtained, which was then flowed for the second purification process using a simple distillation method at a temperature of 80°C for 120 minutes (Fairus et al., 2013). In this process, the mixture was separated based on its boiling point. The condensed xylitol was collected in a vial bottle. The xylitol yield was calculated using the formula in equation 1 below (Fernianti et al., 2020).

$$Yield\ (\%) = \frac{(final\ volume)}{(initial\ weight)} \times 100\% \quad (1)$$

Where final volume is the amount of xylitol produced after purification process, ml, and initial weight is initial mass of raw material (tobacco stem powder) used before processing, g.

2.6 Xylitol content analysis

The sample with the highest yield was tested for its content using High Performance Liquid Chromatography (HPLC) method. The test standard used was from the research Mudrikah et al (Mudrikah et al., 2024). The peak area is used to calculate the concentration of the compound based on the calibration curve that has been made from the standard solution. After obtaining chromatogram data in the form of peak areas of samples and standards, the xylitol yield concentration was then calculated using Equation 2 (Tumanduk et al., 2023).

$$C_s = \frac{(\frac{A_s}{A_{std}} \times C_{std} \times V_t)}{mg} \quad (2)$$

Where C_s is sample concentration (g/L), C_{std} is standard concentration (ppm), A_s is peak area of sample, A_{std} is standard peak area, V_t is sample final volume (L), and mg is sample weight (mg).

2.7 Statistic analysis

Design Expert was used to statistically optimize the number of experiments through the Analysis of Variance (ANOVA) method to identify the best model of the variables. The ANOVA method is a statistical technique used to assess the key variables relationship with other variables (Munawiroh et al., 2020). The ANOVA facilitates the analysis of multiple sample groups while minimizing potential errors, as well as determines whether the average differences between sample groups are significant (Valentino & Lubis, 2021). The analysis was carried out by considering a number of statistical parameters, such as model significance parameters including F-value and p-value, along with coefficient of determination (R^2) to explain variation in the data (Diantoro et al., 2022). These parameters are applied to determine the suitable model explains the correlation between the independent variables and the observed response (Dewi et al., 2023).

The analysis steps were carried out using Design Expert software, starting from inputting experimental data, selecting a model, evaluating the significance of the model through ANOVA, and analyzing statistical parameters (R^2, adjusted R^2, predicted R^2) to determine optimum conditions. The optimum results obtained from the combination of these variables are then shown through ANOVA analysis and statistical parameters to ensure their accuracy. The mathematical model employed to predict the optimum can take the form of either linear or quadratic, depending on the complexity of process variable and response (Hidayat et al., 2021).

3. RESULTS AND DISCUSSION

Determination of maximum xylitol yield in tobacco stems is done using calculations based on optimized variations of solvent, temperature, time, and volume. Each of these factors influences the others to obtain the yield results. The following results of determining the maximum xylitol yield based on calculations using the formula in equation (1) is shown in Table 2.

Table 2. Yield of xylitol

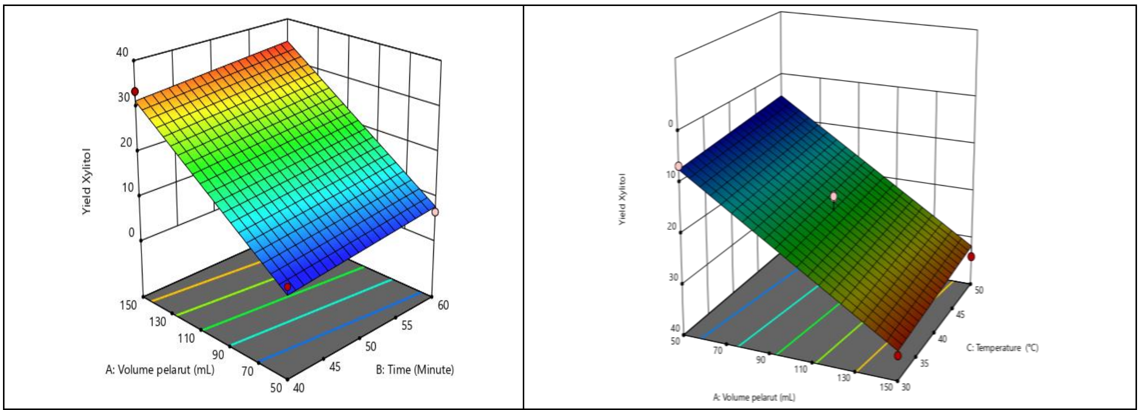
Run	Solvent (mL)	Time (mins)	Temperature (℃)	Yield (%)
1	100	50	40	16.8
2	100	50	40	16.6
3	50	60	40	6.6
4	100	50	40	16.5
5	150	60	40	30.6
6	150	50	50	34
7	50	40	40	6.2
8	100	40	50	21
9	100	40	30	16.2
10	100	50	40	17.1
11	50	50	50	6.2
12	100	50	40	16.7
13	50	50	30	6.8
14	150	40	40	33.4
15	100	60	30	33.2
16	100	60	50	19.8
17	**150**	**50**	**30**	**35.2**

Based on Table 2, the highest xylitol yield obtained is 35.2 % in run 17 at a temperature of 30°C, a time of 50 minutes, and a solvent volume of 150 mL. These conditions are closely related to the effectiveness of the hydrolysis process, which aims to facilitate the production of glucose from lignocellulose using ultrasonic waves in a batch system to produce a cavitation effect. This cavitation can increase mixing and acid penetration, thereby accelerating the hydrolysis process by breaking polysaccharide bonds into simple sugars and increasing glucose concentration, and producing xylitol in more optimal amounts (Purnawan et al., 2021).

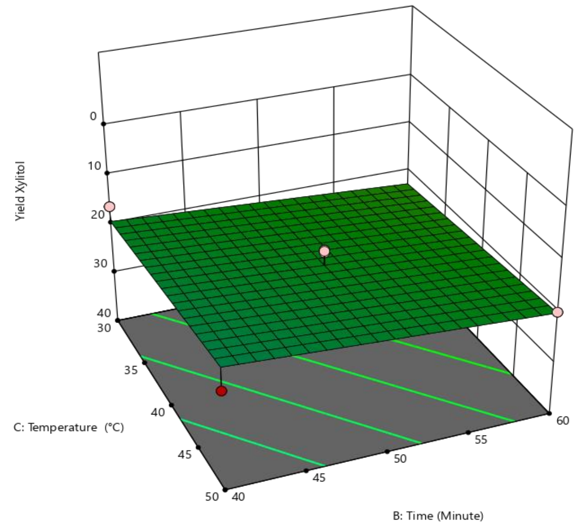
During this process, the use of acid lowers the pH of the solution to a very low level, requiring the addition of base to neutralize it. The neutralization process is carried out by adding NaOH solution, which reacts with H_2SO_4 to form Na_2SO_4 and water. This neutralization is done to achieve the appropriate pH (around 5.5) before the fermentation process. In the fermentation process, the available glucose can be used by *Candida tropicalis* as a source of reducing equivalents needed to reduce xylose to xylitol, which supports cell maintenance and growth. The reaction that occurs can be seen in equation 3 (Fairus et al., 2013).

$$\text{Xilan}(C_5H_8O_4)_{(s)} \rightarrow \text{Xilosa}(C_5H_{10}O_5)_{(aq)} \rightarrow \text{Xylitol}(C_5H_{12}O_5)_{(aq)} \quad (3)$$

The next step is distillation, which is the process of separating two liquid mixtures based on their boiling points (Snyder et al., 2011). In this study, the yield of xylitol produced depends on several variablesm, such as time, temperature, and solvent volume, which serve as tobacco stems.



(a) (b)



(c)

Figure 2. Effect of operating condition: (a) solvent volume and time, (b) solvent volume and temperature, (c) time and temperature on yield

As presented in Figure 2.a., both volume of solvent and processing time affect the amount of xylitol produced, where increasing solvent volume and extending processing time result in higher production. On the other hand, if the solvent volume and time are smaller, the xylitol yield will also decrease. longer contact time increases the diffusion of xylitol molecules into the solvent phase. A significant solvent volume reduces local saturation so that extraction takes place more efficiently (Chaturvedi et al., 2022). Research by Chaturvedi et al shows that in biphasic extraction, the solvent to material ratio and process duration are very important: a larger solvent volume and longer time allow optimal xylitol transfer from fermentation to solvent, so that the yield increases to 79% recovery.

In Figure 2.b., it is shown that the volume of solvent and temperature affect the yield of xylitol obtained. The greater the volume of solvent and the higher the temperature used, the yield tends to increase. Conversely, the smaller the volume of solvent and the lower the temperature used, the resulting yield will also be lower. At low temperatures, the solubility of xylitol decreases, encouraging crystal formation. Optimal solvent volume also helps distribute xylitol evenly and avoids local aggregation, thereby increasing yield and purity (J. Zhang et al., 2022). According to J. Zhang et al, in crystallization using methanol as an antisolvent, temperature and solvent concentration have a significant effect, that higher temperatures and solubility can increase crystallization up to 74% yield with high purity.

In Figure 2.c., it can be seen that time and temperature affect the amount of xylitol produced. Xylitol yield increases at lower temperatures with longer processing times, whereas higher temperatures, in range of 30–40 °C combined with shorter durations lead to a decrease in yield. Previous research stated that extended hydrolysis times allow more xylose from hemicellulose degradation, thereby providing greater xylitol production (Delgado Arcaño et al., 2020). Hilpmann et al., (2020) showed that increasing temperatures accelerate the rate of hydrolysis reactions, thereby increasing the release of xylose from biomass. However, temperatures that are too high (>140 °C) can cause xylose degradation into by-products such as furfural. High temperatures applied too quickly can produce xylose in a short time, but degradation will occur into fermentation inhibitor compounds (Hilpmann et al., 2020).

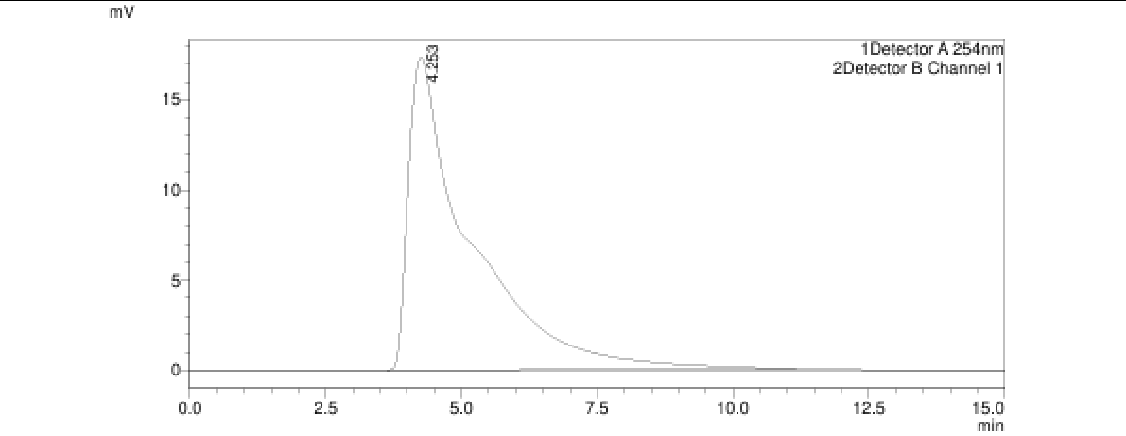
Table 3. Statistical analysis results

Source	Sum Of Squares	Df	Mean Square	F-Value	P-Value	
Model	1477.81	3	492.60	29.66	< 0.0001	Significant
A-Volume of solvent	1441.85	1	1441.85	86.80	< 0.0001	
B-Time	22.44	1	22.44	1.35	0.2660	
C-Temperature	13.52	1	13.52	0.8139	0.3834	
Residual	215.93	13	16.61			
Lack of Fit	215.72	9	23.97	452.25	< 0.0001	Significant
Pure Error	0.2120	4	0.0530			
Total Cast	1693.74	16				
R^2						0.8725
Adjusted R^2						0.8431
Predicted R^2						0.7563
Adeq Precision						15,2761
Std. Dev.						4.08
Mean						19.58
CV %						20.81

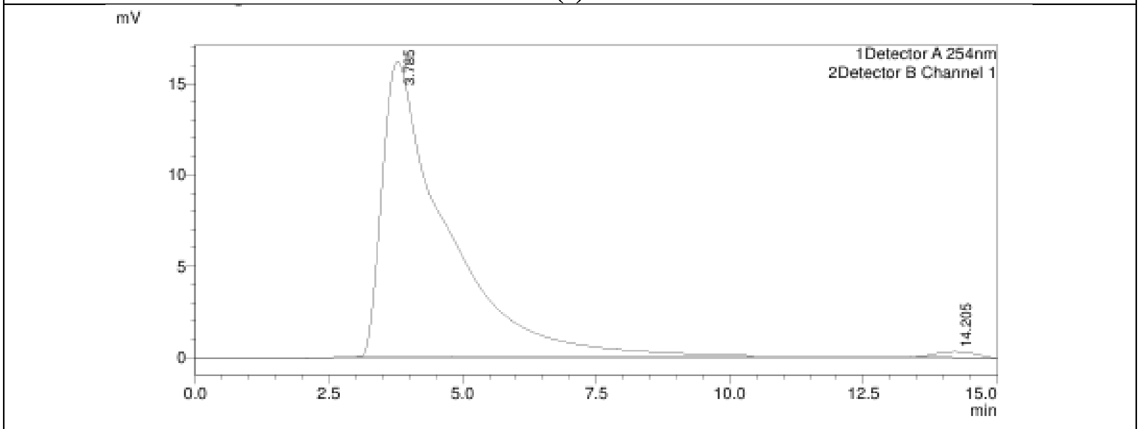
Table 3 show that the F Model value of 29.66 explains that the model proposed by Design Expert is significant, namely linear model. The use of a linear process order model is recommended for statistical analysis of the experimental data obtained through BBD (Hidayat et al., 2021). The parameter is considered significant if the probability value (p-value) ≤ 0.05, whereas p-value > 0.10 indicates no significance. In this study, the p-value of less than 0.05 indicates that the model term is significant. In this case, A is a significant model term. The value greater than 0.10 indicates that the model term is not significant (Ridhuan et al., 2021). This study also reports that according to the selected model, time (B) and temperature (C) are not significant, in accordance with the result presented in Figure 2.c.

Model adequacy is further evaluated through the coefficient of determination ($R^2 > 0.7$) and adequate precision value (>4). The R^2 value ranges from 0 to 1, where values closer to 1 define stronger variable relationships, while lower values reflect weaker associations (Pratiwi et al., 2023). The statistical analysis yielded an R^2 of 0.8725, confirming the model suitability (>75%) (Yulianto & Devina, 2024). A model is considered reliable if the difference between Adjusted R^2 and Predicted R^2 is less than 0.2. In this study, the Adjusted R^2 of 0.8431 shows a strong influence of temperature, time, and solvent volume on xylitol yield production, while the Predicted R^2 of 0.7563 is consistent with the Adjusted R^2, with a difference below 0.2.

The highest yield of xylitol was then tested using the High Performance Liquid Chromatography (HPLC) technique, which was selected due to its efficiency and accuracy (Mudrikah et al., 2024). Then, the concentration of xylitol was measured by analyzing chromatograms with different areas. The larger the area, the higher the concentration of xylitol. also getting bigger. The concentration of xylitol calculated based on the formula in equation 2, results in 5.64 g/L.



(a)



(b)

Figure 3. HPLC result of xylitol content as (a) standard solution, (b) sample

This study shows that the results of utilizing tobacco stem waste into xylitol using the Ultrasound-Assisted Acid Hydrolysis (UAAH) method obtained a xylitol concentration of 5.6 g/L using Equation 2, while in the previous study report that xylitol production from tobacco stalk via sulfuric acid hydrolysis and Candida tropicalis fermentation is 0,18 g/L (Sabancı et al., 2016) and from pineapple through enzymatic using *Candida tropicalis* produced a concentration of 4.29 g/L (Mardawati et al., 2022) . The result obtained by Sabanci et al (2016) has lower xylitol concentration, showed that UAAH performed higher efficiency than conventional acid hydrolysis. The variance in results was attributed to the hydrolysis process, which involves various factors such as solvent concentration, temperature, and hydrolysis time. The yield of xylitol obtained from tobacco stems depends on these factors, which play an important role in maximizing the yield of xylitol.

4. CONCLUSION

This study can be concluded that xylitol in tobacco stems can be extracted using the Ultrasound-Assisted Acid Hydrolysis (UAAH) method, followed by a fermentation process using *Candida tropicalis*. The results obtained indicate that this method is effective in producing xylitol, with the highest yield of 35.2% obtained 30°C, 50 minutes, and 150 mL of solvent. In addition, this study also found that the concentration of xylitol produced from tobacco stem waste with the UAAH and fermentation methods reached 5.6 g/L. This indicates that UAAH coupled with fermentation can be applied to produce xylitol from tobacco stems.

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