



## Production and Characterization of Bioethanol from Tobacco Stalks via Acid Hydrolysis and Fermentation

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### Abstract

The increasing demand for energy and the environmental impacts of fossil fuel consumption have encouraged the development of renewable and sustainable energy sources. Bioethanol derived from lignocellulosic biomass represents a promising alternative fuel due to its renewability and reduced competition with food resources. This study investigates the potential of tobacco stalks, an underutilized agricultural residue, as a feedstock for bioethanol production through acid hydrolysis and fermentation processes. Dried tobacco stalks were hydrolyzed using 1 M sulfuric acid at 110 °C for 3 h to produce fermentable sugars, followed by batch fermentation using *Saccharomyces cerevisiae* with variations in yeast concentration and fermentation time. The fermentation products were purified by simple distillation and characterized using refractometry, density measurement, GC-MS, and bomb calorimetry. The hydrolysis process yielded a sugar concentration of 7.6%. Refractometric analysis indicated ethanol concentrations in the range of 64–68% (v/v), while density measurements suggested lower effective ethanol purity due to residual water and non-ethanol components. GC-MS analysis confirmed ethanol as the dominant compound, with relative contents ranging from approximately 52% to 73%, accompanied by acetic acid and minor volatile by-products. The calorific value of the produced bioethanol ranged from 4,825 to 4,983 kcal/kg and increased with fermentation time. The results demonstrate that tobacco stalks have considerable potential as a lignocellulosic feedstock for bioethanol production, although further process optimization is required to enhance ethanol purity and overall conversion efficiency.

**Keywords:** bioethanol; tobacco stalks; lignocellulosic biomass; acid hydrolysis; fermentation

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## INTRODUCTION

The world is experiencing rapid population growth accompanied by increasing energy demand, while access to energy remains limited in several developing countries (Sharma et al., 2025). Fossil energy sources such as oil, coal, and natural gas still dominate global primary energy consumption (approximately 83%) compared to renewable energy (approximately 12.6%) (Holechek et al., 2022). The use of renewable energy in the electricity, heating, and transportation sectors is projected to increase by nearly 60% between 2024 and 2030, rising from around 13% in 2023 to 20% of final energy consumption by 2030 (IEA, 2024). The utilization of renewable energy sources such as hydropower, solar, geothermal, wind, and biomass is expected to reduce greenhouse gas emissions caused by fossil fuel consumption.

Bioethanol has been widely used in blended fuels such as E10 (10% ethanol, 90% gasoline) and E20. This application has been shown to improve fuel combustion efficiency and reduce exhaust emissions (Hossain et al., 2017). Bioethanol is considered one of the most promising biofuels because it can be produced from a wide range of biomass resources, making it renewable. Biomass feedstocks for bioethanol production include first-generation (1G) biomass derived from food sources (such as sugarcane, molasses, and corn), second-generation (2G) non-food lignocellulosic biomass (such as agricultural residues and wood waste), third-generation (3G) carbohydrate-rich algae, and fourth-generation (4G) genetically engineered algae (Jain & Kumar, 2024). The utilization of 1G biomass may lead to competition with food supply, making 2G biomass more suitable for sustainable development.

Tobacco cultivation generates a substantial amount of agricultural waste, particularly tobacco stalks, which can reach approximately 2.3 tons/ha of dry matter (Saletnik et al., 2024). Tobacco stalks, as an agricultural residue, have significant potential as a bioethanol feedstock. They contain approximately 50% cellulose, 22.6% hemicellulose, and 17% lignin (Handayani & Amrullah, 2018). Cellulose and hemicellulose are polysaccharides that can be hydrolyzed into glucose, which serves as a substrate for bioethanol production. Hydrolysis is commonly performed using acid hydrolysis with sulfuric acid or trifluoroacetic acid. These acids promote saccharification of polymeric chains while minimizing monosaccharide degradation (Bragatto, 2016).

Bioethanol production from lignocellulosic biomass such as tobacco stalks involves several stages, including hydrolysis and fermentation. Acid hydrolysis is commonly conducted using either concentrated or dilute acids. Sulfuric acid concentrations below 4% have been reported to provide relatively high effectiveness at low cost. However, sulfuric acid hydrolysis generates fermentation inhibitors such as furfural and hydroxymethylfurfural (HMF), which reduce fermentation efficiency (Sant et al., 2013). Fermentation is typically carried out using *Saccharomyces cerevisiae*, which effectively converts hexose sugars such as glucose into ethanol (Zhang, 2019). Fermentation still faces several challenges, particularly related to yeast concentration and fermentation duration. Insufficient yeast concentration prolongs fermentation time, increases the risk of microbial contamination, and reduces productivity. Conversely, excessively high yeast concentrations lead to inoculum wastage and do not necessarily result in proportional increases in ethanol yield, making the process economically inefficient (Hashem et al., 2021). Fermentation time is also a critical parameter, as yeast metabolic activity may decline due to ethanol accumulation, nutrient depletion, or environmental stress, resulting in decreased conversion efficiency (Nguyen et al., 2015).

In addition to its energy potential, the valorization of tobacco stalk waste for bioethanol production offers significant environmental and socio-economic benefits. The disposal of tobacco residues is often managed through open burning or uncontrolled dumping, which contributes to air pollution and greenhouse gas emissions while providing no added value to rural economies. Converting tobacco stalks into bioethanol supports the circular bioeconomy by transforming agricultural waste into a value-added renewable fuel, reducing environmental burdens associated with waste management. Moreover, the utilization of locally available biomass residues can enhance energy security, particularly in tobacco-producing regions, by decreasing dependence on imported fossil fuels and creating opportunities for decentralized bioenergy production. These advantages further highlight the relevance of developing efficient and economically viable conversion pathways for lignocellulosic biomass such as tobacco stalks.

This study aims to evaluate the potential of tobacco stalks as a bioethanol feedstock through hydrolysis and fermentation processes and to assess the resulting bioethanol production. The study

focuses on utilizing lignocellulosic tobacco stalk waste, which has not been optimally exploited. In this work, dried tobacco stalks were used as raw material and processed using sulfuric acid hydrolysis. Fermentation was carried out using *Saccharomyces cerevisiae* to convert hydrolyzed sugars into ethanol. This study also includes the determination of bioethanol yield and the evaluation of its energy potential. The results provide an initial assessment of the feasibility of tobacco stalks as a sustainable alternative bioenergy source.

## METHOD

### Materials

The primary material used in this study was tobacco stalks obtained as post-harvest agricultural waste from Wonosobo farming areas. The tobacco stalks were dried at 60 °C for 48 hours. The dried material was then ground and sieved using a 60-mesh sieve to obtain a more uniform particle size. This size reduction was intended to improve hydrolysis efficiency (Yang et al., 2021).

### Acid Hydrolysis and Fermentation

Acid hydrolysis of the tobacco stalk powder was carried out directly using 1 M sulfuric acid ( $\text{H}_2\text{SO}_4$ ) to convert cellulose and hemicellulose fractions into simple sugars (Ubaidilah et al., 2025; Wu et al., 2023). Dilute sulfuric acid at 0.5-2% (w/v, equivalent to ~0.5-2 M) is widely used for hemicellulose hydrolysis in tobacco stalks and agricultural residues, as it effectively solubilizes xylose/glucose while minimizing inhibitor formation compared to concentrated acid. For instance, Hu et al. (2024) achieved 20.3 g/L reducing sugars from tobacco stems using dilute  $\text{H}_2\text{SO}_4$  at 121 °C/90 min, and Zhang et al. (2021) optimized 0.8%  $\text{H}_2\text{SO}_4$  (~0.08 M) presoak for tobacco stalk, yielding high sugar post-explosion.

The hydrolysis process was conducted at a temperature of 110 °C for 3 hours (Dila et al., 2020). These conditions balance hemicellulose degradation with low furfural/HMF formation, aligning with moderate-severity dilute acid pretreatments (100-121 °C, 1-3 h) for tobacco and crop stalks. In previous research, Hu et al. (2024) and Jiang et al., (2024) used similar profiles for biomass, producing 7-20 g/L sugars. After hydrolysis, the reaction mixture was cooled and filtered to separate the solid residue. The resulting filtrate was neutralized to pH 4.8–5.0 prior to fermentation (Woźniak et al., 2025).

The acid hydrolysate was fermented in batch mode using *Saccharomyces cerevisiae* with yeast concentrations of 3%, 5%, and 7% (w/v), supplemented with urea (1.0% w/v) and NPK fertilizer (1.0% w/v). Fermentation was performed at room temperature with fermentation durations of 7, 9, and 11 days. *Saccharomyces cerevisiae* at 3-10% inoculum and 5–14 day batch fermentation maximizes ethanol from lignocellulosic hydrolysates, as validated in tobacco stalk studies (e.g., Zhang et al. 2021; Hu et al., 2024) and general biomass fermentation where longer times accommodate inhibitor effects.

### Ethanol Separation and Purification

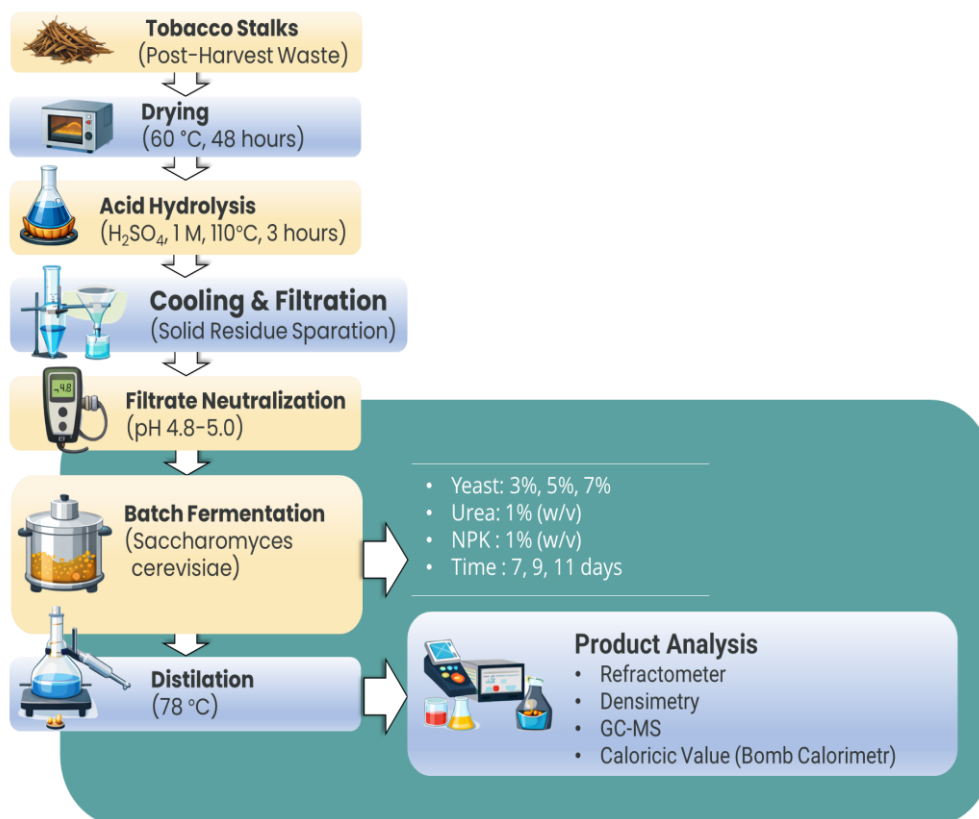
After completion of fermentation, the fermentation broth was separated from solid residues. The liquid product was subjected to simple distillation at 78 °C, close to the boiling point of ethanol. The collected distillate was used as a bioethanol sample for further analysis (Ketut et al., 2025).

### Ethanol Analysis

Ethanol concentration was determined using refractometry, densitometry, and GC-MS analysis, with each method applied for a specific purpose and with consideration of its limitations. Refractometry was used only as a rapid, preliminary estimation method based on changes in refractive index. Although simple and fast, this technique is non-specific and can be influenced by residual sugars, organic acids, and other soluble compounds in the fermentation broth. Therefore, refractometric measurements were not considered reliable for definitive ethanol quantification (Plugatar et al., 2023; Bento et al., 2024).

Densitometry was subsequently applied to obtain a more reliable estimation of ethanol concentration based on the relationship between solution density and ethanol content. This method provides higher accuracy than refractometry; however, it may still be affected by dissolved non-

volatile solids and temperature variations. To reduce these effects, all measurements were conducted under controlled temperature conditions (Plugatar et al., 2023).



**Figure 1.** Production process and analysis of bioethanol from tobacco stalks

GC-MS analysis was used for qualitative confirmation of ethanol presence and identification of volatile compounds. Although gas chromatography is a highly specific technique, quantitative determination requires calibration with external standards. As such calibration was not performed in this study, GC-MS was not used as the primary method for ethanol quantification (Ghazali et al., 2021; Wang et al., 2003; Wang et al., 2023). The calorific value of the bioethanol was determined using a bomb calorimeter to evaluate the energy potential of the produced fuel (Assaye et al., 2021). The overall research flow diagram is shown in **Figure 1**.

## RESULTS AND DISCUSSION

### Preliminary Characterization of Bioethanol

Based on the hydrolysis of tobacco stalks, a sugar concentration of 7.6% was obtained. The hydrolysate was subsequently fermented with fermentation times of 7, 9, and 11 days and yeast masses of 10, 15, and 20 g (**Table 1**). The fermentation products were then distilled to obtain bioethanol fractions, which were analyzed using an ATC 80% refractometer. Refractometric measurements were conducted to obtain a rapid overview of ethanol content prior to more precise analytical methods.

The refractometric results showed ethanol concentrations ranging from 64% to 68% (v/v). At 7 days of fermentation, ethanol concentration increased from 64% to 66% with increasing yeast mass. Fermentation for 9 days produced relatively constant ethanol concentrations of 66% for all yeast variations. At 11 days, the highest ethanol concentration (68%) was obtained using 10 g and 20 g of yeast. These values were used as preliminary estimates, as refractometric measurements are influenced by other components present in the fermentation and distillation products. Potential factors contributing to ethanol overestimation include residual sugars, the presence of other volatile compounds, and bound water affecting the refractive index. More accurate ethanol determination and component identification were subsequently performed using advanced analytical methods.

**Table 1.** Ethanol concentration measured by refractometer

Fermentation time (days)	Yeast mass (g)	Ethanol (%)
7	10	64
	15	66
	20	66
9	10	66
	15	66
	20	66
11	10	68
	15	66
	20	68

**Sources :** The results of this research

### Validation of Ethanol Content Based on Density

To validate refractometric results, bioethanol density was measured using a DMA 4100 density meter following ASTM D4052-22. The density values ranged from 0.8723 to 0.8851 g/cm<sup>3</sup>, as shown in **Table 2**.

**Table 2.** Density of bioethanol produced from tobacco stalk fermentation

Fermentation time (days)	Density (g/cm <sup>3</sup> )	Density (kg/m <sup>3</sup> )
7	6,05764	872.03.00
9	6,06528	873.04.00
11	6,14653	885.01.00

The measured densities were higher than that of absolute ethanol (0.790–0.800 g/cm<sup>3</sup>), indicating that the distilled bioethanol was not fully pure and still contained water and non-ethanol components. The increase in density at 11 days suggests a higher fraction of water or by-products. Compared with refractometric results, density-based estimation provided lower and more conservative ethanol values, serving as a correction for possible overestimation by optical methods. Thus, refractometric data should be considered as baseline information rather than final purity values.

### Compound Characterization Using GC–MS

Dilute acid hydrolysis employing 1 M H<sub>2</sub>SO<sub>4</sub> at 110 °C for 3 h has the potential to induce partial degradation of pentose sugars (predominantly xylose from hemicellulose) and hexose sugars (glucose from cellulose), which may result in the formation of furfural and 5-hydroxymethylfurfural (HMF), respectively. These compounds are widely recognized as fermentation inhibitors that can impair yeast performance and ethanol productivity by affecting cellular metabolic functions. Although the severity of the hydrolysis conditions applied in this study can be considered moderate and comparable to those reported for various agricultural lignocellulosic residues, the formation of inhibitory compounds cannot be ruled out. Nevertheless, furfural and HMF were not quantified in the present work (e.g., by HPLC, as reported by [Świątek et al., 2020](#) and [Godoy et al., 2022](#)), and no detoxification treatments, such as overliming, activated charcoal adsorption, or microbial adaptation, were implemented prior to fermentation. These aspects represent a limitation of the study and should be taken into account when interpreting the fermentation results.

The chemical composition of the bioethanol produced from tobacco stalk fermentation was subsequently evaluated using GC–MS, and the relative abundance of the identified compounds is summarized in **Table 3**. Ethanol was consistently the predominant component under all experimental conditions, followed by acetic acid as the main secondary compound, while several ether compounds were detected only in minor proportions. The occurrence of acetic acid may be associated with the release of acetyl groups from hemicellulose during acid pretreatment and/or with yeast metabolic responses under non-ideal fermentation conditions. In particular, higher acetic



acid levels were observed at extended fermentation times, which may indicate the occurrence of secondary reactions, including partial oxidation of ethanol during fermentation or post-fermentation handling. However, due to the absence of inhibitor quantification, a direct relationship between inhibitor presence and acetic acid formation cannot be conclusively established.

**Table 3.** Bioethanol composition from tobacco stalks

No	Days	Yeast	Ethanol %	Acetic acid %	1,1 Diethoxy methane %	1,1- Diethoxy ethane %	1-Ethoxy- 1- methoxy ethane %	3-Acetyl-2,2- Dimethyl cyclobutane-1- carboxylic acid %
1	7	10	69.09	18.12	0.01	12.77	0.01	-
2	7	15	69.35	17.37	0,02	13.24	0.02	-
3	7	20	70.30	17.07	0.02	12.60	0.01	-
4	9	10	52.32	15.55	0.00	11.47	-	20.66
5	9	15	69.06	17.59	0.01	13.32	0.02	-
6	9	20	69.81	17.44	0.01	12.73	-	-
7	11	10	67.86	18.31	0.02	13.79	0.01	-
8	11	15	72.88	16.37	0.01	10.74	-	-
9	11	20	67.45	18.52	0.01	14.03	-	-

**Sources :** The results of this research

Prolongation of fermentation time generally tended to increase ethanol formation; however, under certain conditions, extended fermentation was also accompanied by increased formation of by-products, notably acetic acid. This trend suggests the presence of an optimal fermentation duration, beyond which further incubation does not proportionally enhance ethanol yield and may instead favor side reactions. Overall, the detection of acetic acid and other minor compounds highlights the need for more comprehensive characterization of hydrolysate composition. Future studies should therefore incorporate systematic inhibitor profiling and evaluate suitable detoxification strategies in order to better elucidate their effects on fermentability and to improve ethanol production efficiency.

### Calorific Value of Bioethanol

Calorific value analysis of the produced bioethanol was conducted using an IKA C200 bomb calorimeter on samples obtained with 10 g yeast at varying fermentation times. As presented in **Table 4**, the calorific value increased with fermentation duration, reaching the highest value at 11 days (4,983 kcal/kg). This trend is consistent with the observed increase in ethanol content at longer fermentation times, as higher ethanol concentrations result in greater energy release during combustion. The calorific values obtained in this study fall within the range reported for bioethanol derived from other lignocellulosic biomass sources, indicating that tobacco stalks are a viable feedstock for bioethanol production. Nevertheless, the relationship between ethanol content and calorific value also highlights that both fermentation efficiency and downstream purification play a critical role in determining the final energy potential of the fuel. Consequently, while tobacco stalk-derived bioethanol demonstrates promising fuel characteristics, further optimization is required to reduce residual water content and co-produced by-products.

**Table 4.** Calorific value of bioethanol produced by fermentation

Fermentation time (days)	Calorific value (kcal/kg)
7	4,825
9	4,851
11	4,983

**Sources :** The results of this research

To contextualize the performance of the present work, **Table 5** summarizes a benchmark comparison of second-generation (2G) bioethanol production, focusing on feedstock recalcitrance, pretreatment severity, and fermentation outcomes. In this study, tobacco stalks were hydrolyzed using 1 M H<sub>2</sub>SO<sub>4</sub> at 110 °C, yielding a hydrolysate sugar concentration of 7.6% and a distillate ethanol relative content of up to 73%. Previous studies have demonstrated that feedstock recalcitrance plays a decisive role in ethanol yield. For example, [Quispe et al. \(2025\)](#) reported that sugarcane bagasse produced 24.20 g/L ethanol under acid hydrolysis at 120 °C, whereas rice husks subjected to identical conditions yielded only 6.85 g/L due to their high ash content. In comparison, the results of the present study suggest that tobacco stalks exhibit a relatively favorable hydrolytic response, even under milder pretreatment conditions (110 °C) than those commonly applied in the literature, such as the 120–121 °C conditions reported by [Quispe et al. \(2025\)](#) and [Vu et al. \(2022\)](#). Moreover, while [Han et al. \(2015\)](#) relied on steam explosion pretreatment to disrupt the fibrous structure of tobacco stalks, the current results indicate that a chemical approach using sulfuric acid with an extended residence time (3 h) can effectively liberate fermentable sugars without the need for high-pressure or specialized equipment.

**Table 5.** Comparative 2G bioethanol from agricultural residues with other references

Feedstock	Method / Conditions	Method / Conditions	Reference
Barley Straw	Acid (H <sub>2</sub> SO <sub>4</sub> ) + Alkaline pretreatment; 121°C (autoclave); ferm. with <i>S. cerevisiae</i> .	Ethanol yield: 16.17 g/L; Total sugar: 205.4 g/L. High sugar release after combined pretreatment.	<a href="#">Vu et al (2022)</a>
Corn Stalks & Leaves	2% NaOH with NiO nanoparticles; 24h fermentation.	Ethanol yield: 15.8 g/L. Nanoparticles acted as biocatalysts to enhance enzymatic hydrolysis efficiency.	<a href="#">Saetang &amp; Tipnee (2022)</a>
Green Coconut Fiber	Alkaline (2% NaOH) pretreatment at 121°C for 1h; enzymatic hydrolysis.	High enzymatic activity (425 U/g). Effective conversion of delignified fiber into fermentable sugars.	<a href="#">Morais et al. (2025)</a>
Rice Husks	Dilute acid hydrolysis (1% H <sub>2</sub> SO <sub>4</sub> ) at 120°C for 1h; Ferm. at 35°C, pH 4.	Ethanol yield: 6.85 g/L. Lower yield compared to other residues due to high silica/ash content.	<a href="#">Quispe et al. (2025)</a>
Coffee Husks	Dilute acid hydrolysis (1% H <sub>2</sub> SO <sub>4</sub> ) at 120°C for 1h; Ferm. at 35°C.	Ethanol yield: 14.73 g/L. Performed significantly better than rice husks under identical acid conditions.	<a href="#">Quispe et al. (2025)</a>
Sugarcane Bagasse	Dilute acid hydrolysis (1% H <sub>2</sub> SO <sub>4</sub> ) at 120°C for 1h; Ferm. at 35°C.	Ethanol yield: 24.20 g/L. The highest yield in the comparative study, confirming bagasse as a superior 2G feedstock.	<a href="#">Quispe et al. (2025)</a>
Tobacco Stalks	Steam explosion & thread rolling; enzymatic hydrolysis (pectinase/cellulase).	Sugar yield: 0.139 g/g. Mechanical/thermal pretreatment significantly exposed cellulose fibers compared to untreated stalks.	<a href="#">Han et al. (2015)</a>
Potato Peel Waste	Enzymatic pretreatment (Laccase); Ferm. with <i>S. cerevisiae</i> .	Reducing sugar yield increased to 1.7 g/L. Demonstrated potential of food processing waste for bioethanol.	<a href="#">Ahmed (2022)</a>
Macroalgae ( <i>L. japonica</i> )	Hydrothermal pretreatment at 50°C; Ferm. with <i>S. cerevisiae</i> .	Bioethanol conc: 1.85 g/L. Separation enhanced by membrane distillation to remove by-products.	<a href="#">Ahmed (2022)</a>
Wheat Straw	Dilute acid hydrolysis (H <sub>2</sub> SO <sub>4</sub> ); Ferm. with <i>P. stipitis</i> (xylose-fermenting).	Ethanol yield: ~19 g/L. Utilizing xylose-fermenting yeast significantly boosts yield from hemicellulose fraction.	<a href="#">Tayyab et al. (2018)</a>
Tobacco Stalks	Acid Hydrolysis (1 M H <sub>2</sub> SO <sub>4</sub> ) at 110°C for 3 h; Batch Ferm. ( <i>S. cerevisiae</i> ); Simple Distillation.	Sugar conc: 7.6%; Ethanol relative content (GC-MS): 52–73%; Calorific Value: 4,825–4,983 kcal/kg.	This study

Despite these advantages, GC–MS analysis revealed a notable co-production of acetic acid alongside ethanol, with relative contents ranging from 52% to 73%. This substantial presence

represents a key trade-off that likely contributed to the observed limitations in calorific value. The formation of acetic acid is attributed to the deacetylation of the hemicellulosic xylan backbone, a reaction that is kinetically favored under the applied 1 M  $\text{H}_2\text{SO}_4$  hydrolysis conditions. While this observation confirms effective biomass deconstruction, acetic acid is known to act as a weak inhibitory compound during fermentation, as it can diffuse across the yeast plasma membrane, lower intracellular pH, and ultimately reduce fermentation efficiency. Furthermore, the measured calorific value range (4,825–4,983 kcal/kg) indicates that the produced bioethanol corresponds to hydrous ethanol below the azeotropic composition. In such systems, residual water functions as a thermal heat sink during combustion, thereby reducing energy density relative to anhydrous ethanol, which typically exhibits a calorific value of approximately 6,400 kcal/kg.

To overcome these limitations, several process improvements are recommended for future studies. First, to mitigate the inhibitory effects of acetic acid and other potential degradation products, the introduction of an overliming detoxification step adjusting the hydrolysate pH to approximately 10 using  $\text{Ca}(\text{OH})_2$  followed by neutralization should be considered prior to yeast inoculation. This approach has been widely reported to precipitate or neutralize inhibitory compounds and improve fermentability. Second, to enhance ethanol purity and calorific value, downstream processing should be extended beyond simple distillation to include advanced dehydration techniques, such as molecular sieve adsorption or pervaporation membrane systems. These technologies are capable of overcoming the ethanol–water azeotrope and producing fuel-grade ethanol with purities exceeding 99%, thereby substantially increasing energy density and suitability for fuel applications.

In addition to downstream upgrading strategies, optimization of hydrolysis severity and fermentation configuration represents a critical pathway to simultaneously reduce acetic acid formation and improve ethanol yield. Lowering acid concentration or shortening hydrolysis residence time may limit excessive deacetylation of hemicellulose while still maintaining sufficient cellulose accessibility. Alternatively, adopting a two-step or sequential hydrolysis–fermentation approach, such as separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF), could reduce sugar degradation and enable more efficient carbon conversion. Moreover, the use of acetic acid–tolerant or genetically adapted yeast strains has been shown to enhance fermentation performance under inhibitory conditions, thereby improving ethanol productivity without extensive chemical detoxification. Collectively, these process-level optimizations highlight that the co-production of acetic acid observed in this study is not solely a limitation but also an opportunity to refine the bioconversion pathway toward higher efficiency and improved fuel-quality bioethanol.

## CONCLUSION

Tobacco stalks were successfully utilized as a feedstock for bioethanol production through acid hydrolysis, fermentation, and distillation processes. Acid hydrolysis produced a sugar concentration of 7.6%, which was subsequently converted into bioethanol with refractometric ethanol concentrations of 64–68% (v/v). Density analysis indicated higher density values than absolute ethanol, confirming the presence of water and non-ethanol components and emphasizing that refractometric measurements are indicative rather than definitive. GC–MS analysis showed ethanol as the dominant compound, with relative contents ranging from approximately 52% to 73%, depending on fermentation conditions. Compared to refractometric measurements, GC–MS results revealed variations in ethanol content, indicating a tendency for refractometric overestimation. Although refractometric values were relatively close to GC–MS results, refractometry may overestimate ethanol concentration because it measures the total refractive index of the solution, which is influenced by non-ethanol compounds such as acetic acid and other volatile components. The calorific value of the produced bioethanol ranged from 4,825 to 4,983 kcal/kg and increased with fermentation time. Overall, the results demonstrate the potential of tobacco stalks as a bioethanol feedstock; however, further optimization is required to improve ethanol purity and process efficiency.



### AUTHOR CONTRIBUTIONS

Conceptualization, AI and EKP; methodology, AM; software, AI; validation, AI, EKP, and AM; formal analysis, AI; investigation, SJ and DW; resources, IM and RJ; data curation, AI; writing—original draft preparation, SJ; writing—review and editing, EKP and AMa; visualization, AI; supervision, EKP; project administration, IM; funding acquisition, AI.

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### CONFLICTS OF INTEREST

The authors declare no conflict of interest concerning the publication of this article. The authors also confirm that the data and the article are free of plagiarism.

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