Fiber Wastes of Date Palm for Bioethanol Production in Saudi Arabia

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A huge rise in energy consumption has been observed in the past few decades because of population and economic growth. One of the renewable energy fuels that can be made from biomass is bioethanol. In Saudi Arabia, date palm provides tons of biomass waste each year, leading to serious problems. The current study aimed to use the date palm fibers (DPF) for bioethanol production *via* a saccharization step (by hydrochloric acid or by *Trichoderma harzianum*) for cellulose. This was followed by fermentation (by *Saccharomyces cerevisiae*). The maximum amount of total carbohydrates (95.55 ± 2.6 mg/dL) and reducing sugar (11.35 ± 0.35 mg/dL) were obtained on the 7th day using *T. harzianum*. The optimum period of bioethanol production was at day 6 (12.52 ± 1.3 g/L), while at day 5 it became (12.76 ± 0.75 g/L) when the DPF were fortified with yeast extract. The bioethanol maximum yield (12.03 ± 1.10 g/L) was obtained at 30 °C. Moreover, 2 mL of *S. cerevisiae* inoculum gave maximum yield of bioethanol. Gas chromatographic analysis showed that bioethanol in the fermented broth represented the major constituent with a peak area of 75.01%. The findings indicate that the fortified DPF with yeast extract gave a promising bioethanol yield.

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Keywords: Fiber wastes; Date palm; Bioethanol; Trichoderma harzianum; Saccharomyces cerevisiae

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INTRODUCTION

Globally, there are over 120 million date palm trees that produce millions of tons of dates each year. Some studies estimate that the waste generated from each tree is over 20 to 35 kg annually. Wastes such as dry leaves, roots, stems, pits, fiber, fronds, and kernels are among the abundant waste products left over from date palms. Lignin, cellulose, and hemicelluloses are the main ingredients of date palm biomass, with little moisture content. Such contents make date palm biomass an attractive resource for conversion of such wastes to bioenergy (Martis *et al*. 2020). As mentioned, Saudi Arabia is considered as one of the greatest producers of dates in the world. Moreover, it is the second-ranked country, yielding 17% dates, represented by 200,000 tons of the total quantity worldwide annually (Hamden *et al*. 2022). Sugars represent the main constituents in dates; such sugars encourage the anaerobic fermentation for the production of bioethanol (Ahmad *et al*. 2021). Because of the date fruit's great nutritional value, health benefits, economic significance, and traditional importance, date palms will continue to be widely planted throughout the Middle East, Africa, and Southwest Asia (Faiad *et al*. 2022). Moreover, the tree plays a great role in the environment *via* absorbing CO_2 to a greater extent as well as storing more

carbon compared to other plants, because of its large mass. Therefore, the date palm may fight to stave off global warming, which is mostly due to emissions of CO_2 (Sharif *et al.* 2010). Betemariyam and Kefalew (2022) documented this ecological role.

After harvesting date fruits, several secondary products from the date plant are collected, such as dry leaves and trunk fibers, which give large quantities of lignocellulosic residues without suitable usage of its byproducts. This renewable resource of wastes could be applied as a substrate for biofuel production as well as bioethanol production (Mehrez *et al*. 2022).

The overconsumption of carbon-based fossil fuels as the main source of energy coincides with rising industrial activity and population (Bateni *et al*. 2014). Utilizing biofuels, namely biomethane and bioethanol, as a substitute to fossil fuels with a considerable reduction in environmental effect is a possibility (Afifi *et al*. 2011; Abdelghany *et al*. 2014; Salama *et al.* 2021). Compared to fossil fuels, the application of bioethanol and biomethane minimizes the emissions of greenhouse gases by up to 86% (Rezania *et al*. 2020).

Microorganisms, particularly yeasts, play a vital role in bioethanol production. Ferreira *et al*. (2019) mentioned that *Saccharomyces cerevisiae* is of great importance in numerous industries such as beverages, food products, and bioethanol production. Yeast, such as *S. cerevisiae,* is utilized in the most processes of alcoholic fermentation, mainly due to its high resistance to ethanol and low optimal range of pH as well as the requirement of anaerobic conditions. According to a recent study, different strains of *Pichia kudriavzevii* were isolated from wastes of date palm fruit (Afolabi and Ola 2022), which provides great yield of bioethanol under fermentation conditions utilizing date palm fruit wastes. According to Sadik and Halema (2014), *Kluyveromyces marxianus*, *Zymomonas mobilis*, and *S. cerevisiae* are superior candidates for production of bioethanol with different levels of productivities at 81.6%, 70.3%, and 88.2%, respectively. Use of date palm fruit wastes with two yeast strains *Pichia kudriavzevii* SGD21 and SGD30 for bioethanol creation might be an efficient method for managing and using date palm fruit wastes (Afolabi and Ola 2022). Residues of date palm are extremely resistant to enzymatic, microbiological, and chemical conversion to biogas. Therefore prior to the generation of biogas, a pretreatment step ordinarily must be carried out to break down the intricate structure of cellulose, remove the lignin barrier, and make it easier for microorganisms or enzymes to penetrate the wastes biomass (Momayez *et al*. 2018).

The term pretreatment means degrading the polymer structure within the biomass so as to accelerate the conversion of polysaccharides to glucose, which will be subsequently fermented to bioethanol (Bagewadi *et al*. 2016; Alotaibi *et al*. 2019). There are different methods for such pretreatment, which include mechanical (pulsed electric field, high-pressure homogenization, microwaves, bead milling, ultrasonication, and, hydrodynamic cavitation), chemical (organo-solvents, acidic alkaline, and oxidative), thermal (freeze thaw cycles and steam explosion), and biological (fungi, bacteria, and enzyme) methods (Nagarajan *et al.* 2020). Most of these pretreatment processes, with the exception of some biological methods, are costly, usually incurring up to 20% of the costs of production (Travaini *et al.* 2016). Some chemical and physical approaches have been studied, such as application of enzymes, acids, microwave, ozone, and ultrasonication. Although great yields have been achieved, some of these methods are expensive, need extreme conditions for the achieved effects, and besides may produce some compounds that prevent the subsequent process of fermentation (Farkas *et al.* 2019). Residues of date palm, such as trunk, leaves, leaf sheath, seeds, and pedicels, have been studied as the basis

for bioethanol production, but only after pretreatment by chemicals or enzymes (Bouaziz *et al*. 2020; Shokrollahi *et al*. 2023).

Trichoderma harzianum was applied in the creation of bioethanol from biomass containing lignocellulose because of its ability to produce cellulolytic enzymes. Pineapple fruit peel was hydrolyzed using *T. harzianum*, giving great yield of fermentable sugar for bioethanol production (Casabar *et al.* 2020). In another study, saccharization of marine macroalgae biomasses was performed by *T. harzianum* prior to hydrolysis by enzymes that enhanced the yield of sugar compared to yield of sugar without fungal pretreatment (Mushlihah *et al.* 2020). Yeasts including *Wikerhamomyces anomalus* and *Pichia stipitis* were applied either in co-cultures or separately for bioethanol production from pretreated date palm fibers by dilute solutions of hydrogen peroxide and sodium hydroxide (Atitallah *et al*. 2022).

The novelty of the present work lies in the use of a selected fungal treatment to achieve saccharization of waste palm fibers, prepared by simple drying and cutting, with no other pretreatment. In this investigation, a saccharification technique using *T. harzianum* was developed. This saccharification technique avoids the production of fermentation-preventing molecules and is ecologically safe with little chemical inputs. Saccharization *via* fungi therefore can be regarded as a promising method for biological treatment, allowing for subsequent fermentation of the sugars to bioethanol. Generally, fungi can create enzymes necessary for the delignification of different biomasses. In so doing, they do not promote problems related to the deactivation of enzymes. In Saudi Arabia, there has been no report about the employment of all parts of date palm for bioethanol production. Therefore, this investigation was designed to assess the potential of converting the date palm fibers to bioethanol *via* saccharification using *T. harzianum.*

EXPERIMENTAL

Materials

Chemicals and microorganisms used

Glucose, absolute ethanol, sulphuric acid, hydrochloric acid, and commercial cellulase were obtained from Sigma-Aldrich (St. Louis, MO, USA). Baker's yeast (*Saccharomyces cerevisiae*) was obtained from Egyptian Sugar and Integrated Industries, Egypt. Using yeast extract peptone dextrose agar medium, *S. cerevisiae* was activated by sub-culturing and incubating at 28 °C for 48 h. *Trichoderma harzianum* was provided by Prof. Tarek M. Abdelghany (Abdelghany and Bakri 2019). All the utilized chemicals in the present investigation were of analytical grade.

Collection Site of Date Palm Fibers

Date palm fibers (DPF) were collected from Al Uyaynah, Diriyah, Riyadh, Saudi Arabia (24°53'03.5"N - 46°27'48.5"E) (Fig. 1) during 2023. The collected DPF were washed using running tap water, and then they were dried at 50 °C in an oven (Borel, Mod. BLN300) to remove water until a constant weight was obtained. Then the material was cut into small pieces.

Fig. 1. Collection site of date palm fibers

Initial Saccharization of DPF

The DPF (the length ranged from 0.5 to 3 cm, while the radius ranged from 0.2 to 1.0 mm) were treated biologically using *T. harzianum* to achieve saccharification of the cellulose content at different incubation periods (3, 6, 9, and 12 days) (Sulaiman 2016). For comparison, saccharification was achieved chemically using hydrochloric acid (HCl). For biological saccharization, 50 g of DPF was mixed with in 500 mL sterilized tap water, then inoculated with two discs (6-mm colony diameter) of *T. harzianum* and incubated for different periods of time. Chemically, the reaction mixture consisted of 50 g of DPF in 500 mL of 6 N HCl was allowed to react for 12 h at 30 ℃.

Total Carbohydrate Detection in the Treated DPF

Total carbohydrate was determined in the treated DPF. The broth was placed in boiling tubes in a water bath for 3 h, and then they were removed and allowed to cool to room temperature. The mixture was neutralized after cooling by adding solid sodium carbonate until effervescence stopped. After adding distilled water to make the entire capacity 100 mL, it was centrifuged, and the supernatant was utilized as a sample.

Working standards of 0.2, 0.4, 0.6, 0.8, and 1 mL of glucose (conc. 0.1 mg/mL) were taken in boiling tubes, and the final volumes of each tube were made 1 mL by adding distilled water. To each tube, 1.0 mL of 5% phenol and 5 mL of 96% sulphuric acid were added and vigorously shaken to ensure that the components were blended properly with the working standard. After 10 min, all of the tubes were submerged for 15 min in a water bath heated to 25 to 30 °C. Using a spectrophotometer, the blank was prepared with 1.0 mL of distilled water, and the optical density (OD) of each tube was measured at 490 nm *via* a spectrophotometer (Model Jenway 6300; Cole-Parmer Ltd., Eaton Socon, England). Then, using 0.2 mL of sample, the entire procedure using the phenol and sulfuric acid addition was repeated, and the ODs of sample solutions were obtained. Moreover, the characterization of DPF for measuring cellulose, hemicellulose, lignin, and ash percentage was performed according to Saadaoui *et al.* (2013).

Reducing Sugar Determination

According to Miller (1959), the reducing sugar content was detected *via* the 3,5-dinitrosalicylic acid (DNS) method. One g of NaOH was added to 70 mL of deionized

H_2O, and then 30 g of Na-K tartrate were added and dissolved in the solution, followed by the addition of 1.0 g of 3,5-dinitrosalicylic acid with continuous stirring. Then, 0.05 g of Na_2SO_3 and 0.2 g of phenol were dissolved. The reaction volume was completed to 100 mL by deionized H_2O and kept in the dark. For detection of reducing sugar, 0.5 mL of sample was mixed with 0.5 mL of DNS solution, followed by boiling for 10 min. The tube containing sample was immediately cooled *via* immersion in cold water. The reaction mixture was fortified with 5 mL of deionized H_2O. The absorbance of the reaction mixture was measured at 546 nm. The reducing sugar quantity was measured from standard curve of glucose.

Bioethanol Production Optimization

The initial saccharization of DPF by *T. harzianum* at optimum conditions of incubation period that gave maximum yields of total carbohydrate and reducing sugar was used for inoculation with *S. cerevisiae* for bioethanol production. The broth of saccharized DPF was inoculated with *S. cerevisiae* at different incubation periods up to 7 days for detecting the optimum incubation period. The DPF supplemented with different concentrations of yeast extract was inoculated with *S. cerevisiae* and incubated at different periods of up to 7 days for detecting the optimum incubation period but with the stimulator yeast extract. The optimum incubation period of maximum yield of bioethanol from DPF fortified with yeast extract (2 g/L), was selected to study the optimum temperature, where DPF was inoculated with *S. cerevisiae* and incubated at different temperatures (10 to 50 ℃) for 5 days. The DPF was inoculated with different inoculum sizes up to 3 mL (0.01 at 600 nm) of *S. cerevisiae* and incubated at 30 ℃ for 5 days to detect the optimum inoculum size. The bioethanol yield was determined at the end of the incubation period of parameter (El-Hussieny *et al*. 2020). Ethanol assay kits were used to determine the quantity of ethanol product *via* colorimetric method at 450 nm.

Bioethanol Detection by Headspace Gas Chromatography

The gas chromatography (GC) technique is a precise and quick technique to identify volatile substances such as ethanol. To identify ethanol in fermented broth in the laboratory, headspace chromatography (HS-GC) (Agilent Technologies, Inc., Santa Clara, CA, USA) was utilized, an approach for identifying volatile components, along with an internal standard technique (ISM). Gas chromatography supplemented with detectors of flame ionization, headspace auto-sampler, and mechanical pipette with capacity 100 µL, volumetric flasks, and appropriate liquid handler were used. The carrier gas used was helium (99.99%) at 1 mL/min flow rate. The temperature of oven during the run was kept at 160 °C, while temperature of 200 °C was specific for both detector and injector port. One µL of fermented distillate broth was injected to HS-GC for investigation. The peak area and retention time of injected fermented distillate broth were documented and compared to the standard compounds to confirm the presence of bioethanol.

Statistical Analysis

All results in triplicate were achieved, and the results were reported as the mean to estimate standard deviation of the obtained findings.

RESULTS AND DISCUSSION

Total Carbohydrates and Reducing Sugars

The collected DPF were treated using *T. harzianum* (Fig. 2) at different incubation periods. The growth of *T. harzianum* was developed using the biomass of DPF. Saccharization of DPF by *T. harzianum* achieved increased total carbohydrate content with increasing incubation period with the maximum amount (95.55 ± 2.6 mg/dL) obtained on day 7, and then the amount decreased on days 9 and 12 to 81.74 ± 1.01 and 77.61 ± 0.53 mg/dL, respectively. In contrast, the chemical saccharification of DPF by HCl showed a high amount of total carbohydrates at 90.77 ± 0.80 mg/dL. Although *T. harzianum* saccharization took a longer time, it avoided the production of inhibitory chemical compounds. Chemical saccharization with HCl is known to prevent or inhibit the fermentation process by yeast, which is required for bioethanol production. Therefore, the current investigation focused on the utilizing *T. harzianum*

The liberated quantity of reducing sugar because of saccharization of DPF by *T. harzianum* differed according to the incubation period (Fig. 3). It reached the maximum amount (11.35 ± 0.35 mg/dL) on day 7, and then it decreased with increasing incubation period up to 12 days. The results were in agreement with other reports (Rani *et al*. 2006; Afolabi and Ola 2022). As mentioned in numerous studies *T. harzianum* is considered an excellent producer of cellulase, which accelerates the fermentation reactions (Evcan and Tari 2015). Therefore, in recent years, investigators have focused on the application *S. cerevisiae* with *T. harzianum* in the bioprocesses, due to better conversion of polysaccharides into glucose, and then conversion to bioethanol (Derman *et al*. 2022). Saccharization of DPF by HCl gave a promising amount of reducing sugar (12.73 ± 0.77 mg/dL) compared to bio-saccharization by *T. harzianum*. However, the delignification of agriculture residues *via* bio- saccharized by microorganisms was achieved with low-energy requirement, slight waste production, and minor environmental conditions. The treated DPF using *T. harzianum* at day 7 that gave a high quantity of carbohydrates and reducing sugar were selected for bioethanol production using *S. cerevisiae* at different conditions. It was found that the cellulose, hemicellulose, lignin, and ash percentages initially were 51.34, 16.56, 11.02, and 2.89%, respectively while they became changed to 23.34, 11.56, 7.02 and, and 2.31%, respectively, as a result of a saccharization step by *T. harzianum*.

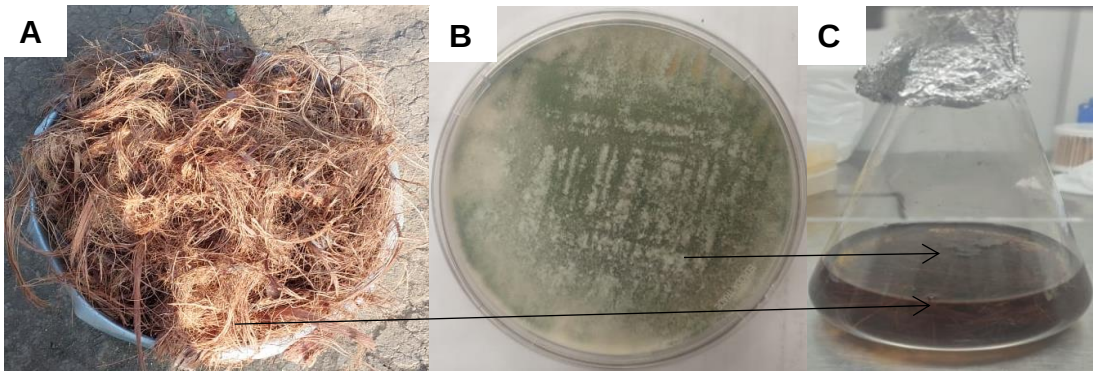


Fig. 2. The collected DPF (A), colony of *T. harzianum* (B), and growth of *T. harzianum* on the DPF biomass (C)

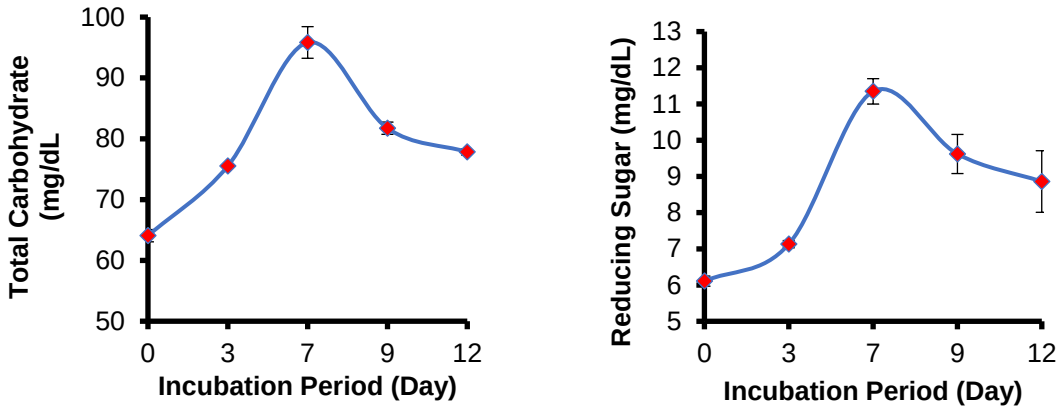


Fig. 3. Total carbohydrate and total reducing sugar content of pre-treated date fibre by *T. harzianum* at different incubation periods

Effect of Different Conditions

Date fibre was initially treated by *T. harzianum* for saccharification at different incubation periods only for obtaining the optimum day at which give maximum total carbohydrate and total reducing sugar, while bioethanol subsequently was produced at different conditions including incubation periods, medium fortified with yeast extracts, temperatures, and inoculum sizes (Figs. 4 to 7). The production of bioethanol by *S. cerevisiae* using pre-treated DPF increased with increasing the incubation period up to day 6 (12.52 ± 1.3 g/L), and then it decreased at day 7 (8.44 ± 1.18 g/L). The addition of yeast extract to DPF encouraged the bioethanol productivity at all tested incubation periods ranging from 1 to 7 days compared to the un-amended DPF. At the same time, the maximum productivity of bioethanol (12.76 ± 0.75 g/L) was obtained at day 5 when DPF was amended with yeast extract (Fig. 5). This correlates with the investigation by Hosny *et al*. (2016) who recorded high yield of bioethanol using amended substrates with yeast extract. There were great differences in bioethanol production based on the incubation temperatures, and 30 ℃ was the optimum temperature for bioethanol production (12.03 ± 1.10 g/L). At 20 ℃, bioethanol productivity was more (9.08 ± 0.10 g/L) than obtained quantity (7.43 ± 1.00 g/L) at 40 ℃ (Fig. 6). Each species has its ideal specific temperature that improves its specific enzymes to stimulate the required reactions. As the inoculum size increased, the bioethanol productivity increased with maximum productivity (12.48 ± 0.5 g/L) at 2 mL inoculum size. Using 1.0 and 1.5 mL of inoculum size, the bioethanol productivity (11.33 ± 0.58 and 11.38 ± 1.06 g/L, respectively) was higher than 2.5 and 3 mL (10.86 ± 0.99 and 9.33 ± 1.53 g/L, respectively) (Fig. 7). Similar observation was recorded in a recent report (Afolabi and Ola 2022), where bioethanol production was enhanced as the concentration of yeast (*Pichia kudriavzevii*) inoculum increased to 30%, but the productivity of bioethanol decreased when the inoculum level increased to 40% using date palm fruit waste. Arslan *et al*. (2021) mentioned that 25% of inoculum concentration is the optimum dose for the bioethanol production. The applied inoculum dose is one of the important factors that affect the lag phase duration, biomass yield, rate of microbial growth, and fermentation process as well as the product yield (de Albuquerque *et al*. 2014). The optimum treatments for bioethanol production are summarized in Fig. 8.

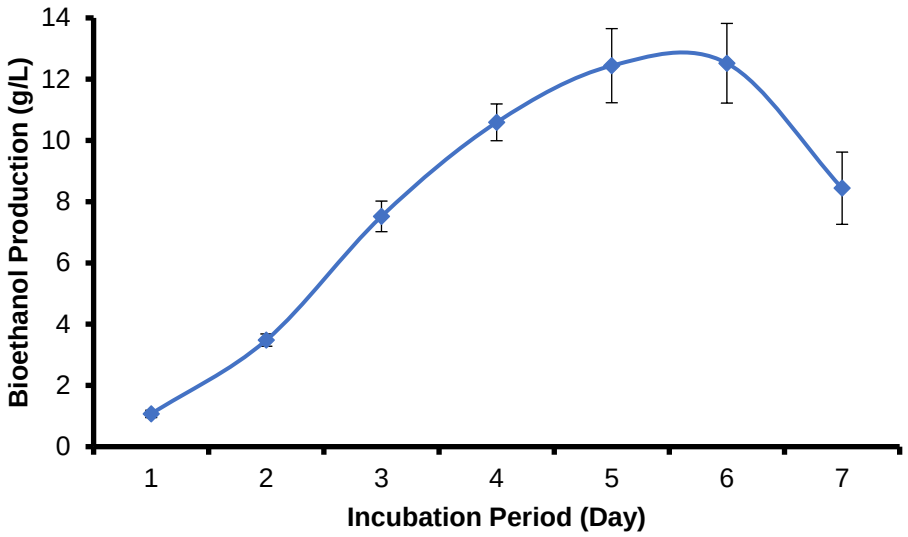


Fig. 4. Bioethanol production at different incubation periods using DPF using *Saccharomyces cerevisiae* at 28 °C

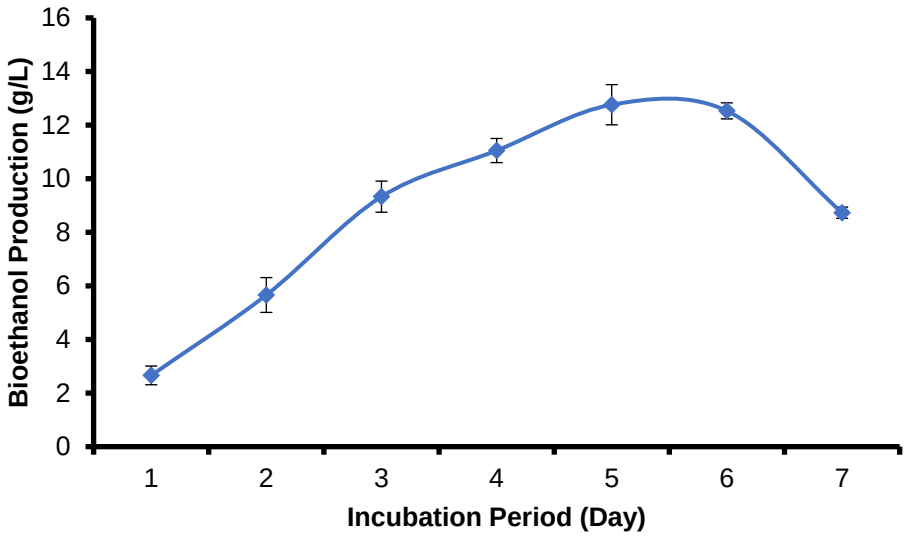


Fig. 5. Bioethanol production at different incubation periods using DPF amended with yeast extract at 28 °C

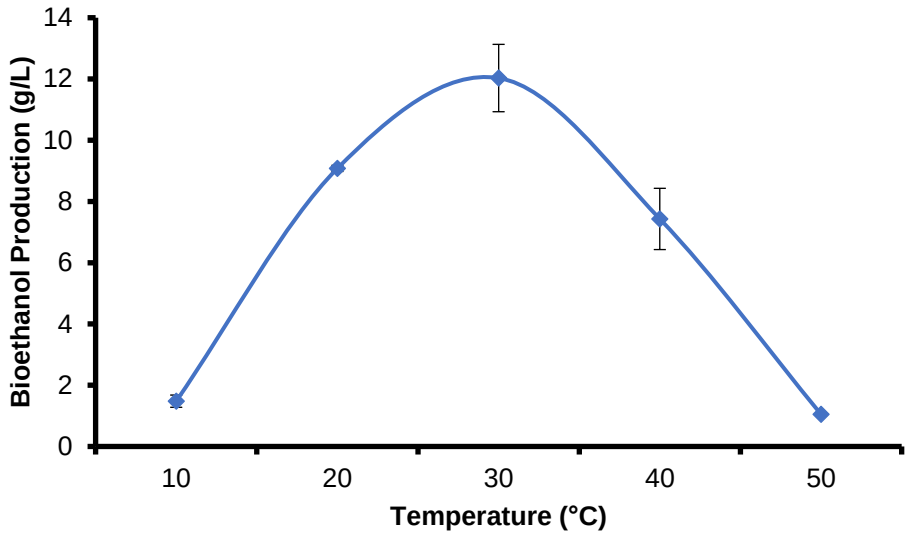


Fig. 6. Bioethanol production at different incubation periods using DPF amended with yeast extract at different temperatures

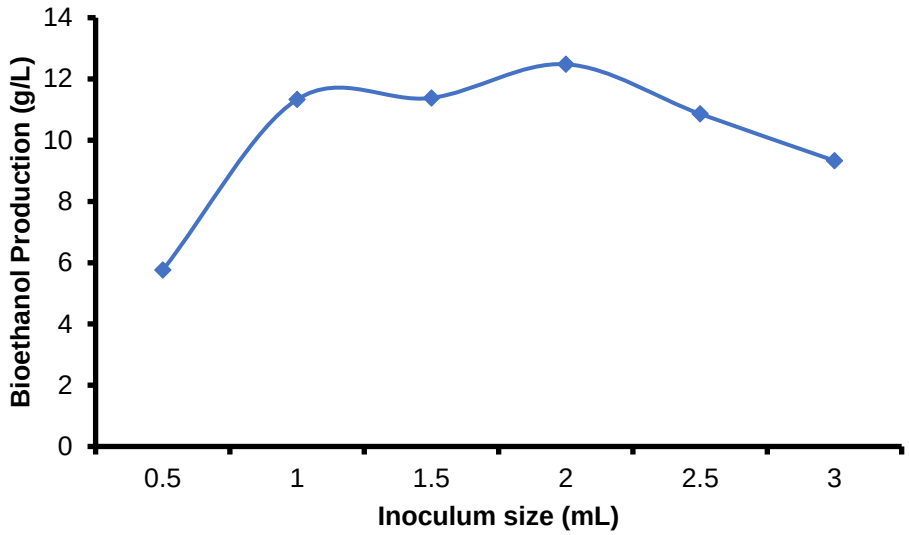


Fig. 7. Bioethanol production using DPF amended with yeast extract using different inoculum sizes of *Saccharomyces cerevisiae* at 30 °C

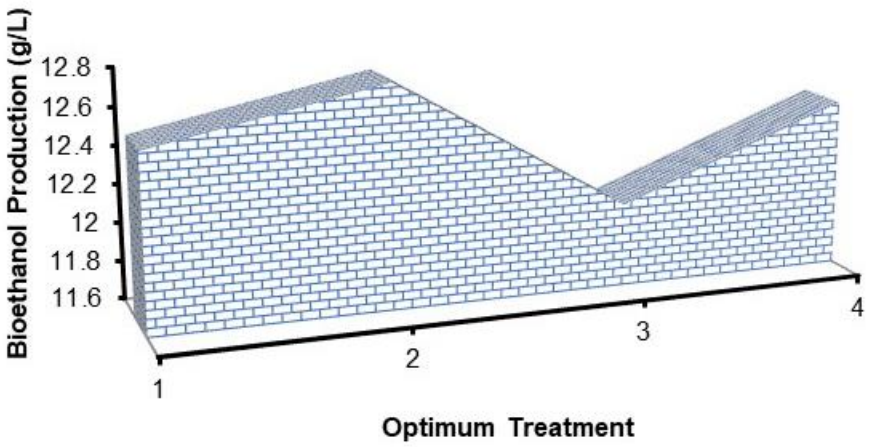


Fig. 8. Summary of the optimum treatments for bioethanol production (1, at 6th day of incubation period; 2, at 5th day of incubation period but the substrate growth amended with yeast extract; 3, at 30 °C; 4, at 2 mL of inoculum size)

In the present study, one sample that exhibited a high content of bioethanol was subjected to bioethanol detection *via* HS-GC analysis. From the analysis, different gases were detected with different retention times (Fig. 9 and Table 1) but according to the area sum %, ethanol represented the greatest productivity with area sum 75.0% , while the other recognized constituents were detected with low area sum percentage. Additionally, the mass spectra of bioethanol are shown in Fig. 10. This study shows that bioethanol yield from DPF was high after fermentation similar to that reported by Antit *et al*. (2021).

Table 1. Detected Ethanol with Other Volatile Constituents in Fermented Broth of DPF

Peak	RT	Name	Formula	Area	Area Sum (%)
1	1.98	Ethanol	C_2H_6O	1532513.15	75.01
2	2.42	2-Propanol	C_3H_8O	129970.16	6.36
3	2.97	Propanoic acid, 2-hydroxy-2-methyl-, methyl ester	$C_5H_{10}O_3$	11681.95	0.57
4	3.67	Chloroform	$CHCl_3$	285865.44	13.99
5	5.37	Acetic acid, propyl ester	$C_5H_{10}O_2$	12051.05	0.59
6	7.60	Nonanal	$C_9H_{18}O$	17786.70	0.87
7	11.80	2-Heptenal, (E)-	$C_7H_{12}O$	12103.95	0.59
8	19.47	2,4-Undecadienal	$C_{11}H_{18}O$	6551.75	0.32
9	19.94	2,4-Decadienal	$C_{10}H_{16}O$	34550.91	1.69

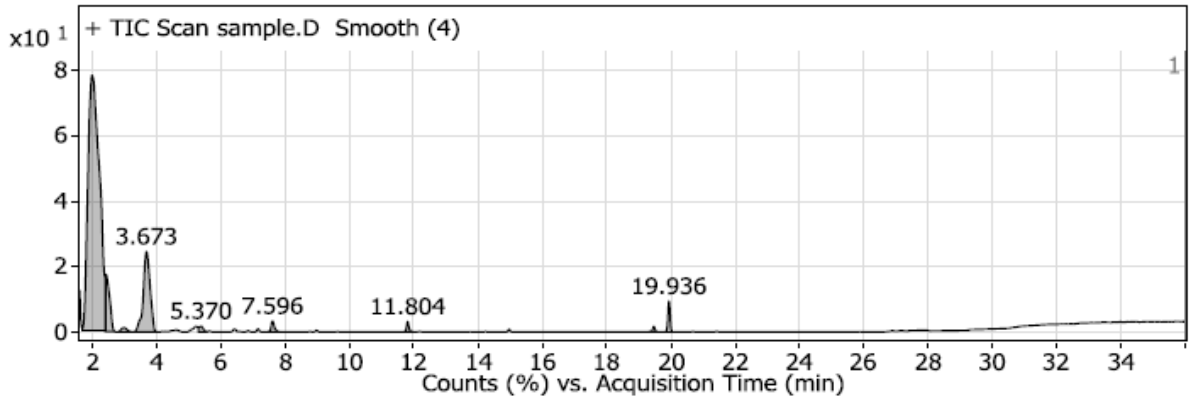
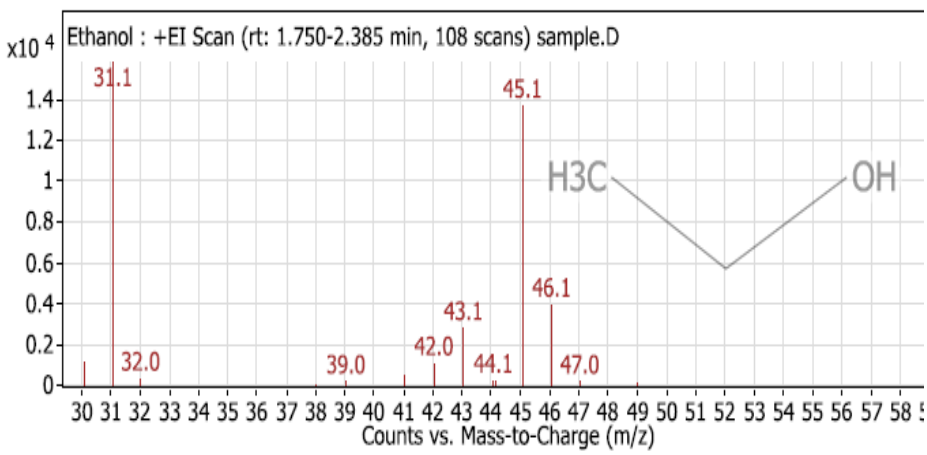


Fig. 9. Chromatogram of headspace gas chromatography of fermented broth analysis for bioethanol (at retention time 1.98) detection with other volatile compounds



Peak List

m/z	Abund
30.1	1087.8
31.1	15825.83
32	323.04
39	172.86
41	470.63
42	1074.44
43.1	2791.74
44.1	217.34
44.2	169.06
45.1	13685.55
46.1	3941.22
47	172.01
59	145.08

Fig. 10. Mass spectra of the detected bioethanol with peak list

CONCLUSIONS

1. The obtained results showed that date palm fiber (DPF) can be regarded as a suitable alternative waste for bioethanol production.
2. The safe fungus *T. harzianum* was effective for the saccharization of DPF, giving good quantity of total carbohydrates and reducing sugar at 7th day of incubation period. The saccharization was achieved without any mechanical or chemical pretreatment.
3. Yeast extract promoted *S. cerevisiae* for bioethanol production from DPF and reduced the optimum incubation period from 6 to 5 days
4. The optimum temperature was 30 °C for bioethanol production from DPF using *S. cerevisiae*.
5. The inoculum size plays a critical role for bioethanol production, where the bioethanol production from DPF decreased at high inoculum size of *S. cerevisiae*.
6. The headspace gas chromatography (HS-GC) analysis appears to be promising for detecting the bioethanol and other volatile compounds.

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