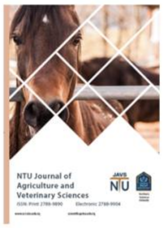
NTU Journal of Agricultural and Veterinary Sciences (2025) 5 (3) : 273-276
DOI: https://doi.org/10.56286/qef1cb29

IRAQI Academic Scientific Journals

JAVS NTU

P-ISSN: 2788-9890 E-ISSN: 2788-9904
NTU Journal of Agricultural and Veterinary Sciences
Available online at: https://journals.ntu.edu.iq/index.php/NTU-JAVS/index



Production Of Distilled Ethanol From Maize and Sorghum Waste Using *Saccharomyces Cerevisiae*

1st Shaima M.SH. Ibrahim, 2nd Fahad K. Y. Al-dulaimi, 3rd Hala A. Abdulrahman
1.2.3 College of Agriculture Technical-Mosul / Northern Technical University.

Article Information

Received: 22-07- 2024,
Accepted: 02-10-2024,
Published online: 28-09-2025

Corresponding author:
Fahad K. Y. Al-dulaimi
College of Agriculture
Technical-Mosul , Mosul,Iraq
Email:Fahadbiologymycology@ntu.edu.iq

Keywords:
Ethanol
Corn Waste
Saccharomyces cerevisiae

A B S T R A C T

The research was conducted in the laboratories of the Agricultural Technical College/Mosul during the period from October 2023. The aim was to produce distilled ethanol from Maize and Sorghum in Iraq. The research included collecting parts (fruits, seeds, and leaves), transferring them to the Agricultural Technical College in the laboratory, and storing them in sterile nylon bags. The fermentation treatments were included 6 treatments to carry out fermentation processes by adding a 2% solution of Saccharomyces cerevisiae yeast to the leaves, fruits, and seeds of corn that were previously prepared and sterilized for fermentation. They were placed in fermenters, and after completing fermentation, carbon dioxide CO2 and ethanol CH3CH2OH were measured, and then the mixture was fractionally distilled to obtain pure ethanol. The results of the productivity experiment treatments after 72 hours showed that the treatment (Maize leaves + 2% yeast) gave the highest production of CO2 gas, 220800 ml, and ethanol, 3.61%, while the treatment (Sorghum fruits + 2% yeast) gave the lowest production of CO2 gas, 131840 ml, and ethanol, 2.15%. After conducting fractional distillation on approximately 15 liters of yeast resulting from this experiment for each treatment, the results showed that the percentage of alcohol distilled was 90%. The highest volume obtained from the Maize leaves treatment was 540 ml, while the lowest volume obtained from the Sorghum fruits + 2% yeast treatment was 322 ml. Based on the results, genetically modified yeast strains can be used to increase efficiency and improve the biofuel production process.

How to cite: Ibrahim, S. M. S., Al-Dulaimi, F. K. Y., & Abdulrahman, H. A. (2025). *Production of distilled ethanol from maize and sorghum waste using Saccharomyces cerevisiae.* **NTU Journal of Agricultural and Veterinary Sciences, 5**(3),

Introduction

In recent decades, the world has witnessed a significant increase in energy consumption, and biofuel has emerged as a promising and sustainable alternative. Biofuel is a source of renewable energy extracted from the fermentation of biomass. It is considered an alternative to traditional fossil fuels (oil, gas, and coal) and is characterized by being more sustainable and less harmful to the environment [1] . It is produced from renewable biological sources, which contributes to reducing greenhouse gas emissions [2]. Iraq relies heavily on oil as a primary source of energy, making it vulnerable to environmental pollution. Biofuel can contribute to diversifying energy sources and reducing dependence on oil as an environmentally friendly energy source, as Iraq has abundant sources of agricultural raw materials that can be used in biofuel production [3] . Maize and Sorghum are important crops in Iraq and the world, as they are characterized by their high content of sugars and starches, the basic raw materials for producing bioethanol. Corn cultivation in Iraq is characterized by being widespread, making it a suitable option for producing biofuel on a large scale [4]. Recent research has focused on producing bioethanol fuel from the cellulosic residues of field crops instead of the starch stored in their seeds, because these residues are cheap, such as straw, hay, bran, wood, etc., compared to the food materials in the seeds of rice, wheat, barley, and corn crops, which are usually more expensive because they are used as staple food for humans and fodder for poultry. This has become the modern general trend in bioethanol production from these cheap lignocellulosic sources [5,6]. *Saccharomyces cerevisiae* yeast plays an important role in the fermentation process, which is the process of converting sugars into alcohol (ethanol) and carbon dioxide in the absence of oxygen. This yeast is one of the most widely used microorganisms in various fermentation processes, thanks to its superior ability to ferment a variety of sugars, including glucose, fructose, sucrose, and maltose [7] . Given the importance of biofuel in the present time, and the urgent need for it in light of the current circumstances in which Iraq relies entirely on fossil fuels, and to preserve the environment from pollution and achieve sustainable development, our study aimed to assess the possibility of producing distilled biofuel (ethanol) from Maize and Sorghum in Iraq and to determine the best varieties of corn crop waste (Maize and Sorghum) in producing the highest percentage of distilled ethanol using *Saccharomyces cerevisiae*.

Materials and methods

Sample collection: Quantities of leaves, fruits, and seeds were collected for each type of Maize and Sorghum. They were placed in sterile nylon bags, numbered, and labeled with specific information for each sample. The bags were tightly sealed and then transferred to the medicinal plants laboratory at the Agricultural Technical College/Department of Plant Production Technologies. The samples were stored in sterile bags at room temperature until testing.

Sample preparation: The samples were cleaned of impurities and dust, then cut and ground to increase the surface area exposed to the yeast.

Yeast solution: Specific amounts of *Saccharomyces cerevisiae* 2% were dissolved in an activation medium to obtain a homogeneous solution.

Fermentation and distillation of ethanol production : The method adopted by CMBTC, 2012 and Palmer, 2006 was used with modifications. A fermenter was used, consisting of a 25 -liter fermentation bottle and a plastic lid that can withstand sterilization temperatures in an autoclave. The plant samples 4 Kg of seeds, fruits, or leaves were mixed with 20 L of water for 3 hours to break down into fermentable sugars. After the decomposition period, the fermenters and their contents were sterilized in an autoclave at 121°C for 15 minutes. After the fermenters cooled to room temperature (25°C), concentrations of the *Saccharomyces cerevisiae* yeast solution were added to each fermenter to start the fermentation process, which was left for 4 days. During this period, the yeast converts the sugars in the corn into alcohol and carbon dioxide [8,9,10,11]. The treatments were divided into sixth treatments based on raw material type:

Treatment 1: (Maize seeds + 2% yeast)

Treatment 2: (Sorghum seeds + 2% yeast)

Treatment 3: (Maize fruits + 2% yeast)

Treatment 4: (Sorghum fruits + 2% yeast)

Treatment 5: (Maize leaves + 2% yeast)

Treatment 6: (Sorghum leaves + 2% yeast)

Measurements during fermentation: During the 72 hrs fermentation period, the following was monitored and measured:

Volume of CO2 gas released from fermentation: CO2 gas was measured using the displacement method [8].

Ethanol estimation: Alcohol was estimated using a refractometer and hydrometer alcohol [12,13].

Fractional distillation : A fractional distillation apparatus was used after the fermentation process was completed and the carbon dioxide gas stopped exiting the fermenter. The yeast was left to settle, Separation of the solid matter (residual fibers) from the liquid (fermented product) using filtration and centrifugation and then the liquid was siphoned off and distilled using the fractional distillation apparatus, where ethanol was separated [13]. The distilled liquid was tested to verify that it was ethanol by several means, including measuring its density using a refractometer, which gives a reading of light refraction. This reading was compared to the refractive index table to determine the percentage of ethanol, as well as using a hydrometer alcohol as mentioned by Nowakowska, 1939 [14]. The ethanol was burned and its odor was smelled. It was also tested with the CrO_3/H_2SO_4 reagent by adding a few drops of this solution and chemically detecting it in the sample of distilled alcohol. The color changed from transparent to greenish-blue, indicating the presence of ethyl alcohol [15,16,17].

Statistical Analysis: The results of the experiments were statistically analyzed after being arranged and tabulated using the Statistical Package for the Social Sciences (SPSS) software according to the Analysis of Variance (ANOVA) test. The means of the treatments were compared using Duncan's multiple range test at a probability level of 0.05 according to Al-Rawi, 2000 [18].

Results and discussion

Carbon dioxide CO2 and ethanol production and distillation

The results in Table 1 for the fermentation treatments after 72 hours and with a yeast volume of 15 liters per treatment, which were obtained after the fermentation processes, showed that the treatment of Maize leaves gave the highest production of CO2 gas, 220800 ml, and ethanol, 3.61%, while the treatment of Sorghum fruits gave the lowest production of CO2 gas, 131840 ml, and ethanol, 2.15%. Our study agreed with many studies, as the reason for the differences is attributed to several factors, including sugar content. Maize leaves contain a higher percentage of simple sugars (such as glucose and fructose) compared to Sorghum fruits, which mainly contain starch, a complex sugar that requires breakdown into simple sugars before the yeast can ferment it. This means that the yeast can easily access the sugars in the Maize leaves and use them to produce ethanol and carbon dioxide. Also, the percentage of fiber in Sorghum fruits is higher than in Maize leaves. Fiber hinders the yeast's access to fermentable sugars, which reduces fermentation efficiency and leads to lower ethanol and carbon dioxide production [16,19,20].

Table 1. Production of CO2 gas and ethanol.

No.	Treatment 2% yeast	Volume co2/ml	Ethanol /ml	Ethanol %	
1	Maize seeds	192220 c	503.35 c	3.14	b
2	Sorghum seeds	164800 d	431.55 d	2.69	c
3	Maize fruits	144000 e	377.08 e	2.35	d
4	Sorghum fruits	131840 f	345.24 f	2.15	d
5	Maize leaves	220800 a	578.19 a	3.61	a
6	Sorghum leaves	208000 b	544.67 b	3.40	a

*Different letters in the same column indicate significant differences at the 5% level.

Maize fruits contain a high percentage of starch, which is a polysaccharide that requires conversion into simple sugars before the yeast can ferment it. Sorghum fruits also contain a high percentage of starch, but may contain a slightly lower percentage of simple sugars compared to Maize . Maize leaves may contain a higher percentage of simple sugars that are easy for yeast to ferment, while Sorghum fruits may contain a higher percentage of complex sugars that take longer to break down before the yeast can ferment them [21,22,23].

Fractional distillation

After conducting fractional distillation on approximately 15 liters of yeast resulting from this experiment for each treatment, the results in Table 2 showed that the percentage of alcohol distilled was 90%. The highest volume obtained from the Maize leaves treatment was 540 ml, while the lowest volume obtained from the Sorghum fruits treatment was 322 ml. The reason for the difference between the treatments in the volume of alcohol produced after fractional distillation can be attributed to several factors, including the initial sugar content. As mentioned earlier, Maize leaves contain a higher percentage of simple sugars compared to Sorghum fruits, which mainly contain starch. This means that Maize leaves provide the yeast with a greater amount of directly fermentable sugar, leading to the production of a larger amount of ethanol and thus a larger volume after distillation. Additionally, the fermentation efficiency of sugars in Maize leaves may be higher than the fermentation efficiency of starch in Sorghum fruits. This is partly because starch requires an additional hydrolysis step by amylase enzymes before the yeast can ferment it, which may lead to the loss of some sugars during this process. Although the percentage of alcohol distilled was 90% for all treatments, it is possible that there was a slight difference in the efficiency of the distillation process between the treatments, leading to a difference in the resulting alcohol volume. Other factors, such as the type of yeast used, fermentation conditions (temperature, pH, aeration), and the chemical composition of Maize and Sorghum, may contribute to the differences in the resulting alcohol volume [16,24,25].

Table 2. Fractional distillation of the ethanol produced.

No.	Treatment 2% yeast	Ethanol /ml concentration 90%
1	Maize seeds	470 c
2	Sorghum seeds	403 d
3	Maize fruits	352 e
4	Sorghum fruits	322 f
5	Maize leaves	540 a
6	Sorghum leaves	509 b

*Different letters in the same column indicate significant differences at the 5% level.

CONCLUSION

The study concluded that using *Saccharomyces cerevisiae* to produce ethanol and carbon dioxide from different corn types (Maize and Sorghum) is a promising approach for biofuel production. The results showed that Maize leaves, when fermented with 2% yeast, produced the highest amount of ethanol and the largest volume of carbon dioxide. However, the production of ethanol and carbon dioxide depends significantly on the yeast concentration and the type of corn part used. The study also indicated that Maize seeds, when fermented with 2% yeast. These findings suggest that the selection of corn type and yeast concentration is crucial for optimizing biofuel production. Furthermore, biofuel production from corn can contribute to achieving sustainable development goals in Iraq by reducing reliance on fossil fuels and providing an alternative energy source. However, further research is needed to assess the economic and environmental feasibility of large-scale biofuel production from corn and to develop more efficient and sustainable technologies.

ACKNOWLEDGMENTS

I would like to express my deepest appreciation to the Department of Plant production techniques at the College of Agricultural Technical / Northern Technical University for there help to improve the quality of this work.

References

[1] Demirel, Y. (2018). Biofuels from Algae for Sustainable Development. In *Algae-Based Biofuels and Bioproducts* (pp. 1-22). Springer, Cham.

[2] Padhan, R. K., Mohanty, B. P., Nayak, S., & Mohanty, K. (2023). Advancement in biofuel production: Recent progress and future perspective. *Fuel*, *332*, 126271.

[3] Hashim, H. M., & Kredy, H. H. (2023). Biofuels in Iraq: Challenges and opportunities. *Energy for Sustainable Development*, *72*, 102339.

[4] Al-Mashhadani, A. H., & Kadhim, A. J. (2020). Production of bioethanol from dates in Iraq. *Journal of Engineering and Sustainable Development*, *24*(4), 1-14

[5] Loow, Y.-L., Wu, T. Y., Jahim, J. M., Mohammad, A. W., & Teoh, W. H. (2015). Lignocellulose biomass as feedstocks for bioethanol production: an overview. *Cellulose*, *22*, 1585–1607. doi:10.1007/s10570-015-0616-7

[6] Meneses, D., Manzanares, P., & Ballesteros, I. (2017). Second generation bioethanol production from lignocellulosic materials: current challenges and approaches taken to improve its environmental impact. *Biofuels, Bioproducts and Biorefining*, *11*(2), 498-516. doi:10.1002/bbb.1761

[7] Tesfaw, A., & Assefa, F. (2014). Current trends in bioethanol production by Saccharomyces cerevisiae: Substrate, inhibitor reduction, growth variables, coculture, and immobilization. *BioMed Research International*, *2014*, 530513.

[8] CMBTC. (2012). The Canadian barley malting and brewing technical guide, 5th Ed. , Canadian Malting Barley Technical Centre , Canada.

[9] Palmer , J.J. (2006). How to Brew. 3 rd Ed. Brewers Publications , USA.

[10] Willey, J. M., Sherwood, L. M., & Woolverton, C. J. (2017). *Prescott's microbiology* (10th ed.). McGraw Hill Education.

[11] Wang, D., Li, Y., & Chen, J. (2010). Optimization of fermentation conditions for bioethanol production from sweet sorghum juice using response surface methodology. *Industrial Crops and Products*, *32*(2), 149-154.

[12] Nelson, D. L., & Cox, M. M. (2017). Lehninger principles of biochemistry (7th ed.).

[13] Fieser, L. F., & Williamson, K. L. (1992). *Organic experiments* (7th ed.). D.C. Heath and Company.

[14] Nowakowska, J. (1939). The refractive indices of ethyl alcohol and water mixtures. MS. Thesis Submitted to Loyola University, Chicago.

[15] Feigl, F. (1966). *Spot tests in organic analysis* (7th ed.). Elsevier.

[16] Bothast, R. J., & Saha, B. C. (1997). Ethanol production from agricultural biomass substrates. *Applied Biochemistry and Biotechnology*, *67*(1), 1-35.

[17] Kim, S., & Dale, B. E. (2004). Global potential bioethanol production from wasted crops and crop residues. *Biomass and Bioenergy*, *26*(4), 361-375.

[18] Al-Rawi, K. M. (2000). *Introduction to Statistics* (2nd ed.). University of Mosul, Ministry of Higher Education Press.

[19] Kim, Y., Mosier, N. S., Hendrickson, R., Ezeji, T., Blaschek, H., Dien, B., ... & Ladisch, M. (2008). Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. *Bioresource Technology*, *99*(12), 5165-5176.

[20] Dien, B. S., Sarath, G., Pedersen, J. F., Sattler, S. E., Chen, H., Funnell-Harris, D. L., ... & Cotta, M. A. (2006). Improved sugar conversion and ethanol yield for forage sorghum (Sorghum bicolor (L.) Moench) lines with reduced lignin contents. *Bioenergy Research*, *1*(2), 153-164.

[21] Wong, J. H., & Man, J. M. (2016). Corn starch: Production, modification, and applications. *Comprehensive Reviews in Food Science and Food Safety*, *15*(6), 1107-1121.

[22] Hui, Y. H. (Ed.). (2006). Handbook of Food Science, Technology, and Engineering. CRC Press.

[23] Lin, Y., & Tanaka, S. (2006). Ethanol fermentation from biomass resources: current state and prospects. *Applied microbiology and biotechnology*, 69(6), 627-642.

[24] Wang, D., Bean, S., McLaren, J., & Wang, H. (2013). Fermentation of corn starch to ethanol by recombinant Zymomonas mobilis. Bioresource Technology, 139, 256-263.

[25] Pandey, A. (2013). *Handbook of plant-based biofuels*. CRC Press.