Journal of Biotechnology Research
ISSN(e): 2413-3256, ISSN(p): 2413-8878
Vol. 6, Issue. 8, pp: 104-108, 2020
URL: https://arpgweb.com/journal/journal/16
DOI: https://doi.org/10.32861/jbr.68.104.108

Academic Research Publishing Group

Original Research **Open Access**

Microbial Fermentation of Water Melon (*Citurullus lanatus*) Seeds for Bioethanol Production

Adegunloye D. V. (Corresponding Author)
Department of Microbiology, School of Science, Federal University of Technology, P. M. B 750, Akure, Ondo State, Nigeria
Email: dvadegunloye@futa.edu.ng

Olotu T. M.
Department of Microbiology, School of Science, Federal University of Technology, P. M. B 750, Akure, Ondo State, Nigeria
Department of Microbiology, Faculty of Science, Adeleke Univers ity, P. M. B 250. Ede, Osun State, Nigeria

Sanusi M. B.
Department of Microbiology, School of Science, Federal University of Technology, P. M. B 750, Akure, Ondo State, Nigeria

Article History
Received: June 6, 2020
Revised: July 18, 2020
Accepted: August 16, 2020
Published: August 20, 2020

Abstract

Watermelon *(Citrullus lanatus)* is a vine-like flowering originally from Southern Africa. The microbial fermentation of watermelon seeds for me production of bioethanol was investigated. The seeds were washed dried and grounded into powder. The seed powder was then fermented for bioethanol production and the microorganisms responsible for the fermentation were isolated and characterized. Bioethanol was distilled from the fermentation. The distilled bioethanol was subjected to comparative analysis with 'the conventional ethanol. Proximate analysis of the grinded seed was carried out before and after fermentation. *The organisms responsible for fermentation were identified as Lactobacillus bulgaricus, Bacillus cereus, Streptococcus lactis, Staphylococcus aureus, Enterococcus* sp, *Micrococcus* spp, *Staphylococcus epidermidis, Fusarium oxysporium, Mucor mucedo, Penicillium notatum, Saccharomyces cerevisiae, Aspergillus fumigatus* and *Aspergillus niger*. There was an increase in the protein content of the seed from 4.269% in the unfermented to 10.031% in the fermented. While carbohydrate reduced from 70.523% in the unfermented to 50.149% in the fermented seed. Fat content increased as well as crude fibre content. The distilled bioethanol boils at 78.4% and as a melting point of -112°C at 15°C. The refractive index was 1.360 and the flash point was 12°C. It burns with blue flame. Considering the comparison between the bioethanol produced from *Citrullus lanatus* seeds with the conventional ethanol, it can be used as an alternative source of biofuel.

Keywords: Biethanol; Fermententation; Microorganisms and *Citrullus lanatus* seeds.

1. Introduction

The environmental changes that have occurred due to the use of fossil fuels have driven the search for alternative sources of fuel that have lower environmental impact. First generation biofuels were derived from crops such as sugar, cane, corn and soybean which contribute to water scarcity and deforestation [1] Fuels that have been extracted from plants and crops are known as biofuels, it is blended with gasoline and can be used as an alternative fuel fuel for vehicles. Plant based fuels which come from renewable sources can be grown anywhere and have lower carbon emission as compared to fossil fuels. Biofuels not only help a struggling economy by providing jobs but also help in reducing greenhouse gases by emitting less pollution. A daily increase in crude oil prices as made most people switch to save money and reduce their dependence on oil [2]. Research has now focused on the non-food crop and waste materials of food crops often discarded by most people all over the world. This is because they are easily accessible, cheap not food competitors and are considered waste. There is need to provide an eco-friendly method for production of bioethanol using watermelon seeds which are generally discarded as food waste. *Citrullus lanatus* (Water melon) is a large, sprawling annual plant with coarse, hairy pinnately-lobed leaves and white to yellow flowers. It is grown for its edible fruit which has a smooth hard rind, usually green with dark green stripes or yellow spots, and juicy, sweet interior flesh usually deep red to pink, but sometimes orange, yellow, or white, with many seeds. A watermelon contains about 6% sugar and 91% water [3]. As with many other fruits, it is good source of Vitamins C and is low in fat and sodium. The seeds have a nutty flavor and can be dried, roasted, or ground into flour. Producing bioethanol without ethical problems such as waste of food resources, as compared to utilization of existing crop biomass or wood-based biomass is the basis of this research and watermelon seeds have the potential for substituting fossil fuel for bioethanol. The aims of this research are to; Isolate and identify the microorganisms responsible for the fermentation of watermelon (*Citrullus lanatus*) seeds, produce bioethanol from water melon seeds by fermentation in distilled water and determine the composition of bioethanol from *citrullus lanatus seeds.*

2. Materials and Methods

2.1. Source and Collection of Sample

Citrullus lanatus **(**watermelon fruits) were obtained at FUTA south gate, Akure, Nigeria. The samples were collected in plastic bags and brought to the laboratory for work.

2.2. Preparation of Seed

The fruits were washed thoroughly under a running tap water to remove sand and dirt. It was then cut open to remove the seed, which were washed clean with distilled water. The seeds were then dried in an oven at 90°C until there was significant change in appearance and it was easy to break. The seeds were milled with a blender after cooling and were sieved with a 40 mesh sieve and stored in air tight bags. The seed powder was divided for the proximate analysis and ethanol production.

2.3. Preparation of Sample

Ten grams of *Citrullus lanatus* powder was introduced into a fermenter and distilled water was added. The initial pH and temperature of the sample was taken. The liquid portion was decanted after 24,48 and 72 hours for bacteria and fungi isolation.

2.4. Determination of the viable Bacterial Count of Samples

Serial dilution of the *(Citrullus lanatus)* powder was made before and after fermenatation, the pour plate method was used. Plates containing Nutrient Agar were incubated in an inverted position at 37°C for 24hours while plates containing Potato Dextrose Agar were ' incubated at 24°C for 72hours. MRS plates were incubated anaerobically. The analyses were done at 0, 24, 48 and 72 hours. All the colonies were counted manually and then multiplied by the corresponding dilution factor.

2.5. Physiochemical and Promate Analysis of *Chrysophyllum albidum* Seed

Moisture content, Ash content, Lipid content, Crude fibre, Crude protein and Carbonhydrate was determined by the difference between the total summations of % moisture, lipids, protein, ash and 100 were all determined using the method of AOAC [4].

2.6. Fermentation

100g of *Citrullus lanatus* seeds was powder and hydrolyzed with 250ml of distilled water; 5g of *Sacchromyces cerevisae* were added and was allow to ferment from 24 to 72hrs

2.7. Ethanol Production

After fermentation the ethanol was produced by using the liquid fraction while was carried out by distillation (fractional distillation).

2.8. Determination of Ethanol

The concentration of ethanol was determined after 24hours for the optimization experiment, the liquid fraction as decanted and distillation carried out (fractional distillation). The ethanol being of a lower boiling point distilled first and is dehydrated using molecular sieves [5].

$$Yield = \frac{gram\ ethanol\ produced\ x\ 100}{gram\ glucose}$$

$$Efficiency = \frac{gram\ ethanol\ produced\ x\ 100}{gram\ glucose\ x\ 0.51}$$

3. Results

3.1. Changes in pH During Fermentation

The pH variations during the fermentation of *Citrullus lanatus* seeds powder is shown in (Table 1). There was a gradual decrease from pH 5.70 to 5.20. This could be as a result of the acid produced by Lactic acid Bacteria making the medium more acidic.

3.2. Changes in the Concentration of Acid During Fermentation of *Citrullus lanatus* Seeds

The initial volume of the acid at 0 hour during titration was 52.83mol/dm. There was a gradual increase in the concentration of acid to 59.57mol/dm^3 at 72 hours as shown in Table 2.

3.3. Microbial Population of Fermented *Citrullus lanatus* Seed

The total microbial population during fermentation is shown in Table 3. The initial bacteria cunt at 0 hour was 5. 0 x 10^7 cfu/ml after 24 hours there was a reduction to 3.0 x 10^6 cfu/ml and 2.10 x 10^6 cfu/ml after 48hours, *then* 1.3 x10^5 at 72hours. The Fungal population increased significantly from 2.0 x 10^4sfu/ml at 0 hour to 6.0 x 10^4 sfu/ml.

3.4. Microorganisms Isolated During the Fermentation of Watermelon Seed

Table 4 shows the bacteria that were isolated during the fermentation of *Citrullus lanatus* seed powder which are: *Lactobacillus bulgaricus, Bacillus cereus, Streptococcus lactis, Staphylococcus aureus, Enterococcus* sp, *Micrococcus* sp, *Staphylococcus epidermidis*, while the fungi isolated which are *Fusarium oxysporium, Mucor mucedo. Saccharomyces cerevisae, Aspergillus fumigatus, Aspergillus niger and Penicillium notatum*

3.5. Proximate Analysis Result

In the proximate analysis of the fermented and unfermented *Citrullus lanatus* seeds, the carbohydrate content decreased through the days as shown in Figures 1 and 2. The protein content, crude fibre content and fat content however decreased.

3.6. Variations in the Bioethanol Produced

There was variation in the bioethanol produced as shown in Table 5. The appearance as well as the refractive index was similar. While the flashpoint, melting point, viscosity varied.

Table-1. pH during the fermentation of *Citrullus lanatus* seeds

Period of fermentation in hours	pH
0	5.70
24	5.40
48	5.30
72	5.20

Table-2. Acid concentration during fermentation of *Citrullus lanatus* seeds

Fermentation in hours	Concentration of acid (mol/dm^3)
0	52.83
24	54.90
48	58.33
72	59.57

Table-3. Microbial population of fermented *Citrullus lanatus* seeds

Fermentation in hours	Fungi (sfu/ml)	Bacteria (sfu/ml)
0	2.0×10^4	5.0×10^7
24	4.0×10^4	3.0×10^6
48	5.0×10^4	2.1×10^6
72	6.0×10^4	1.3×10^5

Table-4. Microorganisms isolated during the fermentation of *Citrullus lanatus* seeds

Bacteria	Fungi
Lactobacillus bulgaricus, Bacillus cereus, Streptococcus lactis, Staphylococcus aureus, Enterococcus sp, *Micrococcus* sp, *Staphylococcus* and *epidermidis*	*Fusarium oxysporium, Mucor mucedo, Saccharomyces cerevisae, Aspergillus fumigatus, Aspergillus niger and Penicillium notatum*

Figure-1. Proximate composition of Citrullus lanatus seeds at 24hrs

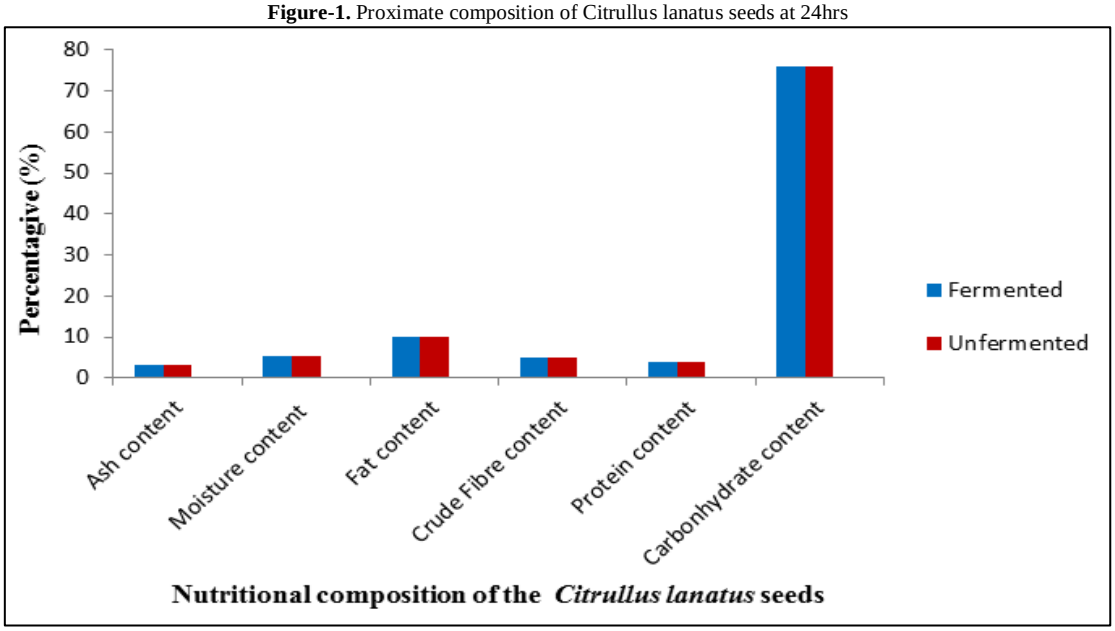

Figure-2. Proximate composition of Citrullus lanatus seeds at 48 and 72 hrs

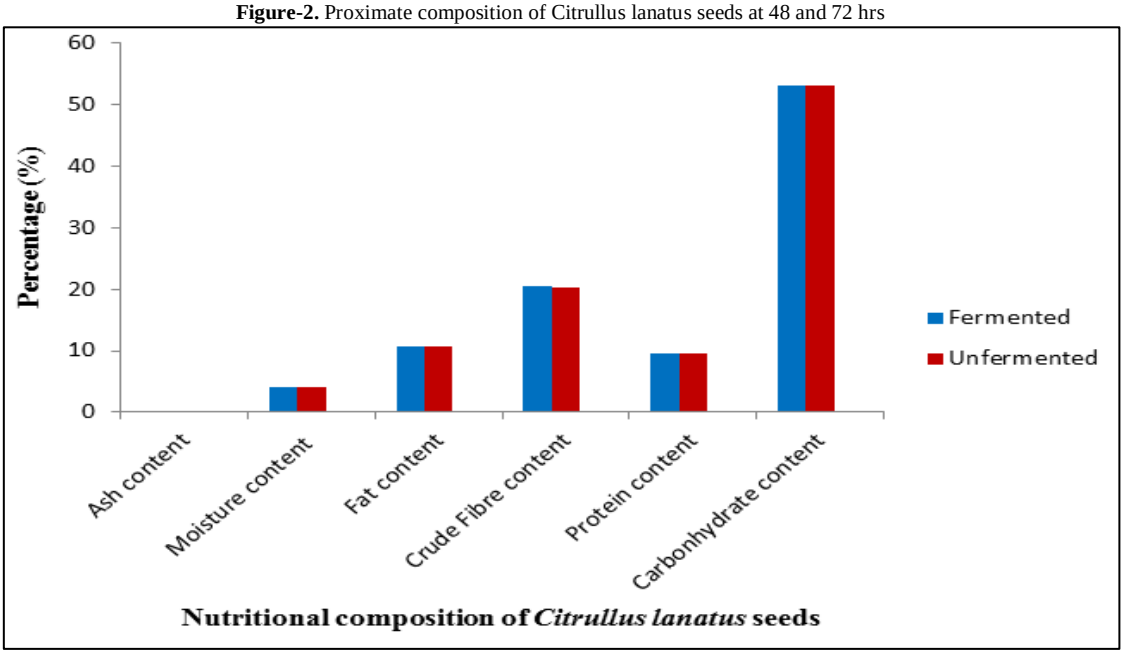

Table-5. Comparative analysis of the *Citrullus lanatus* bioethanol and convectional ethanol

Parameters	Bioethanol	Ethanol
Appearance	Colourless	Colourless
Relative density	0.75g/cm^3	0.789g/cm^3
Melting point	-112°C	-114^{0}C
Boiling point	78.4°C	78.37°C
Viscosity	0.0123	0.0012pas at 20^{o}C
Burning characteristics	Burning with blue flame	Burning with blue flame
Refractive index	1.364	1.362
Flash point	12.5°C	13-14°C

4. Discussion

The initial pH at 0 hour was 5.7 which later decreased to 5.4 at 24 hours, then to 5.3 at 48hours and finally to 5.2 at 72 hours during the fermentation process. The changes in pH may be due to the production of acid, by acid producing microorganisms present in the fermentation process. This decrease could also be as a result of the high carbohydrate content at day 1 which decreased through continuous fermentation where it is converted to fermentable sugars by yeast thereby making the medium more acidic as shown in (Table 1). The initial volume of the acid at 0 hour during titration was 52.83mol/dm^3. There was a gradual increase in the concentration of acid to 59.57mol/dm^3 at 72 hours as shown in (Table 2). There was a change in the medium acidity such that the concentration of the acid increased from 52.83mol/dm^3 at 0 hour to 54.90ml/dm^3 at 24 hours and finally to 59.57mol/dm^3 at 72hours. These have however reduced the useful and available nutrient in the sample [6].

The population of bacteria at 0 hour was high with a count of 5.0 x 10 cfu/ml however the lowest count was recorded at 72hours which was 1.3 x 10^5 cfu/ml as shown in (Table 3). This decrease could be due to low pH which could hinder the growth of some bacteria and also the bacteriocin as well as other chemicals secreted by other organisms to bring about inhibitory effect on other organisms. The presence of *Stapylococcus aureus* at 0 hour could be due to contamination from human skin. Also, the presence of *Bacillus cereus* isolated at 24 hours could be due to exposure of sample during drying to dust. Other bacteria isolated such as *Lactobacillus bulgaricus* and *Streptococcus lactis* were present due to the fact that in the fermentation of *Citrullus lanatus*, lactic acid was produced. The fungal population increased significantly, from 2.0 x 10^4 sfuml at 0 hour to 6.0 x l0^{4}sfu/ml at 72 hours. This is because fungi are known to surviveacidic and other harsh conditions. The isolated bacteria that were involved in the fermentation are show in Table 4. Thebacteria are *Lactobacillus bulgaricus, Bacillus cereus, Streptococcus lactis,aureus, Enterococcus* sp, *Micrococcus* spp, *Staphylococcus epidermidis*, while Table 4 shows the fungi isolated which are *Fusarium oxysporium, Mucor mucedo, Saccharomyces cerevisae, Aspergillusfumigatus, Aspergillus niger and Penicillium notatum*

In the proximate analysis of the fermented and unfermented *Citrullus lanatus* seed, the Carbohydrate content decreased through the days. The protein content, crude fibre content and fat content increased while moisture content however was low in Figures (1 and 2). The bioethanol produced was analyzed and the result quantified as regards the appearance, relative density, meting point, boiling point, viscosity, burning characteristics, flash point and ' refractive index were compared with the conventional ethanol. It burns with blue flame, it is colourless and the refractive index is similar to that of the conventional ethanol as shown in (Table 5). It was observed that the relative density of the bioethanol produced was 0.75g/cm^3 as against 0.789g/cm^3 of the conventional ethanol. It also has a flash point of 12^{o}C and melting point of -112°C. These similarities make it useful as an alternative fuel.

5. Conclusion

The *Citrullus lanatus* seeds are often regarded as waste and are discarded; it has been shown in this research that it can be used for the production of bioethanol through fermentation. The constant search on alternative source of biofuel should be focused on watermelon seed because it combats the idea of food versus fuel. This would also bring about a decrease in the depletion of the ozone layer and expulsion of greenhouse gases which is detrimental to the health of humans.

Reference

[1] Demirbas, A., 2010. *Social, economic, environmental and policy aspect of biofuels*. Energy Edusa Technol. part B., pp. 75-109.

[2] Balat, H., 2009. *Prospects of biofuels for a sustainable future: a critical assessment*. Energy Edusa Technol, pp. 75-111.

[3] Rimando and Perkins-Veazie, 2005. "Determination of citrulline in water melon rind." *J. Chromotograph A.*, vol. 110, pp. 196-943.

[4] AOAC, 2005. *Official methods or analysis*. 14th ed. Washington D.C: Association of Official Analytical Chemist.

[5] Frederic, W. J., Lien, S. J., Courchene, C. E., Dermatini, N. A., Ragauskas, A. J., and Lisa, K., 2008. "Production of Bioetahnol from carbonhydrates from loblolly pine; A technical and economic assessment." *Bores Tech.*, vol. 99, pp. 5051-5057.

[6] Shiota, A., Hotta, Y., Kataoka, T., Monta, M., Maeda, Y., and Kimurak, J., 2013. "Sex med. Oral cituilline Supplementation improves Erectile function in rats with Acute Arteriogenic Erectile dysfunction." *J. Sex. Med.*, vol. 10, pp. 2423-2429.