Ajiboye and Olawoyin, Pan African Journal of Life Sciences (2024): 8(2): 131-137

Publication of Faculty of Basic Medical Sciences and Basic Clinical Sciences , Ladoke Akintola University of Technology, Ogbomoso

www.pajols.org
Online ISSN:2672-5924

RESEARCH Open Access

Comparative Studies of Bioethanol Produced from Cassava Peel Using *Aspergillus niger* and *Saccharomyces Cerevisiae* in Submerged Fermentation

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Received 26th December 2023; Revised 23th April 2024; Accepted 24th April 2024

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ABSTRACT

Background: Cassava peel is one of the largest agricultural wastes in Nigeria, and it is generated by processing cassava tubers for human and industrial use. This project study compared the bioethanol produced from cassava peel using *Saccharomyces cerevisiae* ATCC 204508/S288c and *Aspergillus niger* isolated from onion bulbs.

Methods: Proximate analysis was done on the peels using standard methods of analyses of the Association of Official Analytical Chemists (AOAC). Fermentation for bioethanol production was done for 6 days using submerged fermentation. Parameters such as ethanol yield, substrate concentration and pH were optimised for bioethanol production. The pH was determined using standard techniques. Ethanol yield was measured via spectrophotometer and ethanol standard curve techniques.

Results: Proximate composition showed the following: moisture (9.75± 0.55 %), crude ash (3.12± 0.25 %), crude fibre (9.50± 0.01 %), crude fat (1.50±0.05 %), protein (2.54± 0.06 %), carbohydrate (73.50 ± 0.05 %). pH ranged from 4.50±0.25 to 3.20±0.30 for *S. cerevisiae*, and *A. niger* ranged from 4.50±0.30 to 3.20±0.04. Ethanol yield for *S. cerevisiae* ranged from 18.50±0.05b to 28.03±0.45a with a concentration of 30 g/ml having the highest at Day 4 and 30 g/ml having the lowest at Day 1 and *A. niger* ranging from 17.00±0.50b to 27.54±0.05b with concentration 30 g/ml having the highest at Day 4 and 10 g/ml having the lowest at Day 1.

Conclusion: This research, therefore, indicates that cassava peel could serve as a cheap and promising substrate for bioethanol production using *S. cerevisiae* and *A. niger*.

Keywords: Bioethanol, Cassava peels, *Saccharomyces cerevisiae, Aspergillus niger*

1.0 INTRODUCTION

Bioethanol production by hydrolysis and fermentation of starch is frequently used to create drinkable alcohols like beer, whisky, and other distilled spirits, as well as biofuel, medicinal, and cosmetic ethanol. At the start of the 20th century, the United States was the first country to produce fuel ethanol from starch [1]. Bioethanol is a microbiological process that converts simple sugar into ethanol and carbon dioxide (CO_2) [2]. It is a primary fuel that can be used as an alternative to gasoline for vehicles [3] and a renewable energy source created mainly by sugar fermentation. However, it can also be produced chemically by combining ethylene with steam [4]. Bioethanol is a byproduct of microbial fermentation, as opposed to synthetic ethanol produced from petrochemical sources. It is created through the fermentation of sugars in biomass and utilised as a biofuel or in combination with petroleum in internal combustion engines [5].

Ethanol may be produced from food industry waste at a reasonable cost and without affecting the environment. They contain a complex mixture of cellulose, soluble sugar, carbohydrates, lipids, proteins, and other elements. They provide inexpensive fermentable ingredients for bioethanol creation rather than being thrown away [6]. Their utilisation in this way also eliminates the issues of dumping them in landfills or burning them [7]. Cassava is the primary energy crop and one of the renewable resources utilised for bioethanol production. Cassava starch can be used on large scales to produce ethanol in tropical countries, where Thailand is one of the largest cassava producers in the world [8]. It is a renewable carbohydrate carbon source and available in abundance. It is cheap, clean, non-toxic, and widely used as an essential feedstock for various industrial applications, especially for the production of ethanol [9]. Cassavas are Nigeria's primary raw material for ethanol production, whereas sugar cane is the primary source in Brazil [10]. *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Aspergillus niger* and *Schizosaccharomyces* pombe, are microorganisms able to convert sugars to ethanol [11]. In Nigeria, cassava processing to make different food products results in the annual disposal of large amounts of cassava garbage. Surface water and groundwater quality are threatened when this garbage is dumped in open areas or through drainage systems. Therefore, it is essential to transform these wastes into beneficial end products rather than leaving them to pollute the environment [12].

In addition to reducing greenhouse gas emissions and other pollutants and the political and economic vulnerability that comes with them, biofuels are anticipated to stimulate the economy by driving up demand and prices for agricultural products [13]. The demand for bioethanol as an alternative energy source is rising, and Nigeria currently imports ethanol to meet its domestic demand. Biofuels like bioethanol and biodiesel benefit significantly from the rising need for cleaner transportation fuels. In this work, comparative studies of bioethanol produced from cassava peels by *Aspergillus niger* and *Saccharomyces cerevisiae* using submerged fermentation was done.

2.0 METHODOLOGY

2.1 Sample Collection/Preparation

The cassava peels were collected from Oja Oba market Ilorin, Kwara State, Nigeria, and samples were aseptically collected into a clean polythene bag. It was sorted and washed under running tap water to remove sand and other impurities. They were air-dried at room temperature for two weeks. After drying, it was pulverised into powdered form with the aid of a Binatone blender (Binatone blender model: BLG-452), sieved with a fine mesh of 0.05mm in diameter, and stored in an air-tight container for further use.

2.2 Pretreatment of Cassava Peels Using Acid Hydrolysis

Pretreatment of cassava peel was done using dilute H_2SO_4. Thirty grams (30 g) of cassava peel were weighed and placed into a separate 500 ml conical flask, and 250 ml of dilute H_2SO_4 was added to a conical flask containing the sample. It was then autoclaved at 121 ^oC and was filtered using Whitman filter paper. The residue was washed for 30 minutes until a pH of 7 was achieved, and it was oven-dried at 100 -105 ℃ [14].

2.3 Proximate Analysis

This was done on the cassava peel to determine its nutrient composition. The proximate composition was determined using the standard analysis methods of the Association of Official Analytical Chemists [15].

2.4 Test Organism

Dry baker's yeast (*Saccharomyces cerevisiae* ATCC 204508/S288c) was obtained from the Department of Microbiology, University of Ilorin. Cultural and microscopic characterisation was done to confirm the spherical and

oval budding cells [16]. *Aspergillus niger* was isolated from onions bulb due to its relative humidity and storage temperature, which ranges from 33±2 °C, which promotes the growth of *A. niger*. Colony morphology, cellular characters, and molecular identification of the fungal isolates were done.

2.5 Inoculum Preparation

2.5.1 *Aspergillus niger*

Spore suspensions of the fungal isolates were prepared by adding 10 ml of sterilised distilled water containing two drops of 0.1 % tween 80 to the sporulated seven-day-old culture. A sterile wire loop was used to dislodge the spore clusters under sterile conditions, and then the clusters were mixed thoroughly to prepare a uniform spore suspension. The number of spores $2.0x10^5$ spores per 1 ml were counted using Neubauer's counting chamber as described by Singh *et al.* [17].

2.5.2 *Saccharomyces cerevisiae*

Two grams (2g) of dry baker's yeast was grown on a yeast peptone dextrose (YPD) agar plate at 30 °C for 48 h to activate the yeast. A loopful of the yeast colony was transferred from the agar plate to 100 ml of 5 % YPD broth (which was obtained by dissolving 5 grams of the broth in 100 ml of solution using distilled water) and incubated at 28±2 0C on a shaker (Stuart Orbital Shaker SSL1) at 130 rpm for 48 hours. Precisely Seven (7 ml) of the broth was centrifuged at 4500 rpm for 5 min. The supernatant was decanted, the pellet (deposits) was resuspended in 10 ml of sterile distilled water twice, centrifuged, and the supernatant decanted. The pellet was resuspended in 1 % 50 ml citrate buffer and was used as inoculum [16].

2.6 Bioethanol Production by Submerged Fermentation

Bioethanol fermentation was carried out by submerged fermentation in 250 ml cotton wool plugged Erlenmeyer flasks containing the hydrolysed sample and autoclaved at 121 ^oC for 15 minutes. The samples were allowed to cool at 28±2 °C. Cultures of *Aspergillus niger, Saccharomyces cerevisiae* and a combination of *Aspergillus niger* and *Saccharomyces cerevisiae* were aseptically inoculated into each flask containing the pretreated samples. The flasks were corked using cotton wool, shaken and incubated for 6 days. The flasks were shaken at intervals to produce a homogenous solution and even distribution of the organisms in the substrate mixture. After every 24 hours, the samples were aseptically withdrawn from the fermentation medium and centrifuged at 4,500 rpm for 6 minutes. pH and ethanol yield were determined using the filtrate obtained with the spectrophotometer and ethanol standard curve assay method [17].

2.7 Optimization Parameters for Bioethanol Production

2.7.1 Effect of Substrate Concentration

Different grams of pretreated samples 10, 20, 30, 40, and 50 grams were weighed and dispensed into a Mineral Salt Medium and 100 ml of Distilled Water, which was autoclaved and cooled. An aliquot of fungi spore suspension (1 ml) containing $2.0x10^5$ spores/ml was inoculated into each flask and incubated at 30 °C on a rotary Shaker at 200 rpm for 6 days [17].

2.8 Physicochemical Analysis

2.8.1 Assays for pH

The pH of each sample during the six days of fermentation was determined every 24 hours using a pH meter (pH meter model: OHAUZ STARTAR 2000). The pH meter was first calibrated with acid and base before use. Five ml of each solution was pipetted into a 200 ml conical flask, and the electrode was dipped into it. The pH of the samples was read and recorded [14].

2.8.2 Assays for Bioethanol Yield

Five (5 ml) of the fermenting broth was measured into a test tube, and then 2 ml of the prepared potassium dichromate solution was added to each shaken thoroughly and allowed to stay for 20 minutes. The solution in the test tube was poured into a labelled cuticle in the ultraviolet (UV) visible spectrophotometer and analysed to determine the wavelength and percentage of ethanol concentration, which was extrapolated from the standard ethanol curve [18].

2.9 Recovering Bioethanol through distillation

The bioethanol produced was separated from the water using fractional distillation. Since the boiling point of bioethanol is 78.4 ^0C while that of water is 100 ^0C, the mixture was maintained at 78.4 ^0C, a temperature at which the bioethanol was vaporised in a distilling flask. The vapour, mostly bioethanol, was trapped in the condenser connected to the fractionating column of the distilling flask. The vapour was later condensed back to ethanol and was received in a round-bottom flask. Thus, the bioethanol produced was recovered after the sample was fermented [19].

2.10 Data Analysis

The data were statistically processed to estimate the mean ± standard deviation (SD), and the two-way analysis of variance (ANOVA) was used for comparison. All data were analysed using the Statistical Package for Social Sciences (SPSS) version 19.0 (SPSS Inc., Chicago, IL). A P value of < 0.05 was considered to be statistically significant.

3.0 RESULTS

3.1 Proximate Composition of Cassava Peels

The proximate composition of cassava peels showed mean percentage moisture of 9.75±0.55, a percentage of ash of 3.12±0.25, a percentage of carbohydrate 73.50±0.05, a percentage of lipid 1.50±0.05, a percentage of crude fibre of 9.50 ± 0.01 and crude protein 2.54±0.06 as seen in Table 1.

Table 1. Proximate Composition of Cassava Peels

Parameter (%)	Mean ± SEM Values
Moisture	9.75± 0.55
Ash	3.12± 0.25
Carbohydrate	73.50 ± 0.05
Lipid	1.50± 0.05
Crude fibre	9.50± 0.01
Crude protein	2.54± 0.06

Values are means of triplicate readings and ± standard deviation of proximate composition of cassava peels

3.2 Macroscopic and Microscopic Characteristics of Fungal Isolate

Macroscopic observation of fungal isolates (F_1-F2) is shown in Table 2. F1 revealed that their growth was initially white but changed to black after a few days, producing conidial spores. The edges of the colonies appear pale yellow, producing radial fissures. F2 reveals that it was smooth, moist, cream, glistening, and had thread-like hyphae. A microscopic view of (F1) revealed smooth-coloured conidiophores and conidia. The conidiophores are protrusions from a septate and hyaline hypha. The conidial heads appear radial, and they split into columns (biseriate), and it was identified as *Aspergillus niger*. F2 revealed that the buds are tiny at first, then they enlarge progressively and eventually separate from the mother cell, and it was identified as *Saccharomyces cerevisiae*.

3.3 pH during fermentation of Cassava Peels to Produce Bioethanol at Different Substrate Concentration

Table 2: Morphological Characterization of Fungal Isolates

Isolates	Macroscopic and Microscopic Description	Probable Organisms
F_1	Whitish colonies appear black after a few days, filamentous in nature, and conidial production and the reverse is pale yellow. Hyphae are septate; conidial heads are radiated and split into columns at maturity. Conidiospores are long, smooth and hyaline. Conidial are brown to black and very rough.	*Aspergillus niger*
F_2	Smooth, moist, cream, glistening and has thread-like hyphae. Buds are small at first but enlarge progressively and eventually separate from mother cells by forming cross walls with a large central vacuole.	*Saccharomyces cerevisiae*

Table 3. pH during Fermentation of Cassava Peels to Produce Bioethanol at Different Substrate Concentrations

Sub. Conc. (g/100 ml)	Fungal Isolates	Fermentation Period (Days) / pH value					
		1	2	3	4	5	6
10	S. C	4.50±0.25^a	4.40±0.32^a	3.20±0.30^b	3.70±0.26^a	3.35±0.20^a	3.70±0.25^a
	A. G	4.00±0.21^b	4.00±0.05^a	3.70±0.01^a	4.00±0.05^a	3.57±0.26^b	3.55±0.18^a
20	S. C	4.20±0.06^a	4.10±0.05^a	3.80±0.01^a	3.50±0.05^a	3.80±0.25^b	3.50±0.30^a
	A. G	4.50±0.30^a	4.42±0.05^a	3.40±0.05^b	3.20±0.04^b	3.50±0.26^b	3.37±0.25^b
30	S. C	4.00±0.05^a	4.10±0.06^a	4.20±0.01^a	3.60±0.50^a	3.40±0.05^a	3.20±0.06^b
	A. G	4.50±0.01^b	4.00±0.06^a	3.70±0.22^a	3.50±0.30^a	3.55±0.05^a	3.40±0.55^b
40	S.C	4.20±0.05^b	4.00±0.35^a	3.50±0.23^b	3.80±0.05^a	3.90±0.66^b	3.50±0.05^a
	A. G	4.00±0.05^a	3.80±0.01^b	3.50±0.06^b	3.50±0.22^b	3.53±0.05^b	3.42±0.01^b
50	S. C	3.80±0.06^b	3.50±0.05^b	3.55±0.03^b	3.50±0.06^b	3.45±0.06^a	3.40±0.06^b
	A. G	4.00±0.05^a	3.80±0.06^b	3.70±0.55^a	3.65±0.05^b	3.55±0.25^a	3.24±0.05^a

Values are means of triplicate readings ± standard deviation of pH of cassava peels using varying substrate concentration values on the same column with different superscripts are significantly different at $P < 0.05$. Key: S.C – *Saccharomyces cerevisiae*; AG - *Aspergillus niger*

The pH value obtained from fermentation cassava peels using Saccharomyces cerevisiae at different substrate concentrations ranged from 4.50±0.25a to 3.20±0.30b, with a concentration of 10 g/ml having the highest on Day 1 and 10 g/ml having the lowest on Day 3. Fermentation using Aspergillus niger ranged from 4.50±0.30a to 3.20±0.04b, with a concentration of 10 g/ml having the highest on Day 1 and 20 g/ml having the lowest on Day 4, as shown in Table 3.

3.4 Bioethanol Yield during Fermentation of Cassava Peel to Produce Bioethanol at Different Substrate Concentration

The bioethanol yield value produced using Saccharomyces cerevisiae at different substrate concentrations ranged from 18.50±0.05b to 28.03±0.45a with concentrations 30 g/ml having the highest on Day 4 and 30 g/ml having the lowest on Day 1. The bioethanol yield value produced using *Aspergillus niger* ranged from 17.00±0.50b to 27.54±0.05b, with a concentration of 30 g/ml having the highest on Day 4 and 10 g/ml having the lowest on Day 1, as seen in Table 4.

Table 4. Ethanol Yield During Fermentation of Cassava Peel to Produce Bioethanol at Different Substrate Concentration

Sub. Conc. (g/100 ml)	Fungal Isolates	Fermentation Period (Day) / Ethanol yield (%)					
		1	2	3	4	5	6
10	S. C	18.50±0.05^{b}	19.80±0.40^{b}	22.00±0.01^{a}	23.50±0.05^{a}	23.21±0.06^{a}	22.50±0.50^{a}
	A. G	17.00±0.50^{b}	17.68±0.05^{b}	20.65±0.02^{b}	21.67±0.01^{b}	22.90±0.05^{a}	21.89±0.45a
20	S. C	19.32±0.65^{b}	20.70±0.05^{a}	22.50±0.05^{a}	23.40±0.01^{a}	23.90±0.23^{a}	23.53±0.25^{a}
	A. G	18.25±0.01^{b}	19.54±0.07^{b}	21.55±0.05^{b}	23.00±0.01^{a}	25.67±0.50^{b}	25.55±0.30^{b}
30	S. C	20.50±0.05^{a}	24.05±0.05a	26.50±0.06^{b}	28.03±0.45^{a}	28.00±0.56^{b}	27.45±0.76^{b}
	A. G	18.20±0.02^{b}	19.90±0.05^{b}	21.90±0.03^{b}	25.55±0.01^{a}	27.54±0.05^{b}	27.50±0.06b
40	S. C	21.56±0.30^{a}	22.05±0.50^{a}	24.60±0.05^{b}	23.60±0.08^{b}	23.80±0.34^{b}	23.55±0.01^{a}
	A. G	22.00±0.05^{a}	22.50±0.56^{a}	23.90±0.30^{a}	25.56±0.45^{a}	26.00±0.09^{b}	25.55±0.06^{b}
50	S. C	21.70±0.06^{a}	22.90±0.05^{a}	23.65±0.01^{a}	23.70±0.05^{b}	22.65±0.40^{a}	22.60±0.50^{b}
	A. G	22.10±0.01^{a}	22.26±0.23^{a}	23.00±0.44^{a}	24.60±0.23^{b}	25.12±0.50^{b}	25.00±0.40^{b}

Values are means of triplicate readings ± standard deviation of ethanol yield of cassava peels using varying substrate concentration values on the same column with different superscripts are significantly different at $P < 0.05$. Key: S.C – *Saccharomyces cerevisiae*; AG - *Aspergillus niger*.

4.0 DISCUSSION

Bioethanol production is generating considerable interest worldwide due to its immense significance and use as a subclass of biofuels. According to a recent research [20], lignocellulose and cellulose are the primary biomass sources for bioethanol synthesis. Cassava peels were the substrate of choice for this study.

From the result, the sample's moisture content was very low, which was lower than the report of Akpabio *et al.* [21] that compared the moisture content of fresh peels of two different cassava cultivars. This may be because the moisture content was determined by using fresh peels. The ash content provides an estimate of the quality of the product. The result obtained in this study showed that cassava peels have a very low ash content. This indicates cassava peels, especially the macro minerals, may not have high mineral content. The result obtained for crude fibre showed that cassava peels have relatively low crude fibre content. The result was higher than the 8.47 % reported by Adeolu and Enesi [22] for fresh plantain bract. It was also low compared to the results of Akpabio *et al.* [21], who reported varieties of two cassava peels. The lipid content of the sample used in this study was slightly lower than that of Adeolu and Enesi [22], who reported a low lipid content for fresh plantain bract. The protein content of the sample was low. The low protein content might be due to the high carbohydrate content of the sample [21]. The carbohydrate content of the sample was relatively high, and the value may have been high because cassava is a carbohydrate-rich food. The carbohydrate content was higher than that of Adeolu and Enesi [22], who reported a carbohydrate content of 60.87 % in plantain bract waste.

The sample's pH fluctuates during the 5-day fermentation period. As the pH decreases, the fermenting broth becomes more acidic, thus changing the organisms' metabolic activities for increased ethanol production. The acidic pH recorded during fermentation, however, enhances ethanol production and also serves as a deterrent to bacterial contaminants [18]. This finding is similar to that of Nester *et al.* [23]. A decrease in pH also resulted

in a substantial increase in bioethanol production.

The fermentation of the substrates using *Saccharomyces cerevisiae* showed that bioethanol yield is proportional to fermentation time until after four days when there was a drop, while the yield increased with an increase in fermentation time. This correlation exists due to continuous utilisation of the sugar by yeast, which agrees with the findings of Chen *et al.* [24]. The highest ethanol yield was achieved at a substrate concentration of 30 g/100ml and decreased beyond this concentration. The decrease in ethanol yield above this concentration may be due to the inhibitory effect of the higher substrate concentration on yeast cells. This is similar to a result reported by Swain *et al.* [25].

At high substrate concentrations, the pH of fermenting broth inoculated with *Aspergillus niger* was higher than that of Saccharomyces cerevisiae. This report, however, does not agree with the work of Yan *et al.* [26], which reported that an increase in pH resulted in a substantial decrease in bioethanol production and the growth of microorganisms. According to the result, at a pH of 4.50, there was low bioethanol production, presumably because the low pH encourages acid production instead of alcohol. This could be due to the formation of acetic acid, which inhibits bioethanol production. This observation agrees with the findings of Jennifer *et al.* [27]. However, the bioethanol production from S. cerevisiae was higher than that of *A. niger*. Previous published results have indicated that the fermentation time dramatically influences the bioethanol production by yeasts [28].

The result of this study confirmed that bioethanol can be produced from cassava peel, which is agricultural waste. Despite the ability to use cassava peel for bioethanol production, the yield can be influenced by several factors, especially pH, fermentation period, and substrate concentration. The use of *S. cerevisiae* and *A. niger* is suitable for the production of bioethanol from cassava peels.

Conflicts of Interest

The authors declare that there is no conflict of interests.

Authors' Contributions

AAE conceived and designed the study and contributed to data analysis tools. **ORA** performed data collection, contributed to data analysis tools, analysis of data and manuscript writing. All authors approved the final copy of the manuscript.

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