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Effect of Incubation Period and Temperature on the Bioethanol Yield Produced from Cassava Peel by *Aspergillus niger* and *Saccharomyces cerevisiae* in Submerged Fermentation Process

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ABSTRACT: Various agrowastes have been found to be useful in bioethanol production. Microbial activity during the fermentation cycle results in the bioconversion of simple sugars into ethanol and carbon dioxide (CO_2). Large-scale ethanol production has been achieved worldwide using cassava starch. Thus, the objective of this paper was to evaluate the effect of incubation period and temperature on bioethanol yield from cassava peel by *Saccharomyces cerevisiae ATCC 204508/S288c and Aspergillus niger KC 329626 in submerged fermentation process*. The physicochemical parameters which include the pH, TTA and specific gravity were assayed to determine the optimal condition for the bioethanol yield at different temperature and incubation period. At a substrate concentration of 30 mg/100 ml, *Saccharomyces cerevisiae* recorded the highest value of bioethanol yield (29.56 ± 0.05) and *Aspergillus niger* (26.52 ± 0.07) at day 4, while the lowest yields were obtained on day 1 for *Aspergillus niger* (19.00 ± 0.50) and 20.88 ± 0.04b for *Saccharomyces cerevisiae* of the bioethanol production process. The recorded mean values for SG (kg/m^3) were close, the pH and reducing sugar decreased as the incubation period (days) increases, while the TTA (ml) and the ethanol output (g/ml) also increased. At day 4 of the production series, the optimum temperature at which the maximum bioethanol yield was recorded was found to be at 30 °C. This study thus revealed that cassava peel may be processed and utilized as a alternative source for commercial and industrial bioethanol production as long as the proper conditions are met for the optimization of the fungal strain used.

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The production of bioethanol from starch was first introduced at the beginning of the twentieth century (Cripwell *et al*., 2020). Bioethanol is an attractive alternative fuel because it is an eco-friendly renewable bio-based resource that contributes to the reduction of fuel emissions from petroleum oil, thereby reducing the associated negative environmental impacts generated by the worldwide utilization of petroleum oil (Siqueira *et al*., 2018). Moreover, the hydrolysis and fermentation of starch to bioethanol is widely used for the production of biofuel, pharmaceutical and cosmetic ethanol, and other ethanol products. The use of bioethanol as liquid fuels, particularly for transportation (Tesfaw and Assefa, 2014), as well as its prospective use in industrial processes and heating (Balat and Balat, 2018), makes it appealing. Comparing bioethanol to traditional liquid fuels reveals advantages. It has been demonstrated that switching from gasoline to ethanol reduces carbon emissions by 80% (Madeira *et al*.,

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2017). Simple sugar is converted by microbes into ethanol and carbon dioxide (CO_2) in the process of producing bioethanol (Agulejika *et al*., 2015). This primary fuel can be used to replace gasoline in vehicles (Pakula and Pentella, 2010). It is also a renewable energy source that is mostly made by fermenting sugar, while it can also be made chemically by reacting ethylene with steam (Kroumov*et al*., 2018). Fuel or energy crops such maize, cassava and cassava products, wheat crops, waste straw, guinea corn husk, rice husk, millet husk, sawdust, sorghum plant, sugar cane, and sweet potatoes are the primary sources of sugar needed to make ethanol (Kim and Dele, 2005; Balat *et al*., 2018). One of the renewable resources used to produce bioethanol is cassava, which is also the main energy crop. Microorganisms that may convert carbohydrates to ethanol include *Schizosaccharomyces pombe*, *Aspergilus niger*, *Zymomonasmobilis*, and *Saccharomyces cerevisiae* (Obadina *et al*., 2006). The third-largest source of carbohydrates for human consumption worldwide is cassava, a crop that is widely grown for food. The majority of these wastes and residues have a respectable proportion of cellulose, which is a suitable source of fermentable sugars for substantial applications. Given that Nigeria is a large agricultural nation, disposing of agricultural waste will seriously pollute the environment in addition to being expensive. In our previous published work, we observed that aside the period of incubation and the temperature requirements, the hydrogen ion concentration could also be a contribution factor to the quality and yield of the bioethanol (Ajiboye and Olawoyin, 2024). Therefore, the country should support the technology that turns these wastes into money (bioethanol). Thus, the objective of this paper was to evaluate the effect of temperature and incubation period on bioethanol yield from cassava peel by *Saccharomyces cerevisiae ATCC 204508/S288c and Aspergillus niger KC 329626* in a submerged fermentation process.

MATERIALS AND METHODS

Sample Collection: The sample used for this study cassava peel, was procured at an indigenous market in Ilorin, the capital of Kwara State, Nigeria. The cassava peels were aseptically gathered in sterile Ziploc bags and immediately transferred to the microbiology laboratory of the institution for further analysis.

Preparation of cassava peel for bioethanol production: In order to get rid of sand and other contaminants, the gathered cassava peels were sorted and cleaned under running water. For two weeks, they were allowed to air dry at room temperature. After drying, it was ground into a powder using a Binatone blender (model number BLG-452) and sieved through a fine 0.05 mm mesh screen before being kept in an airtight container for later use.

Test Organisms: Dried baker's yeast of *Saccharomyces cerevisiae* ATCC 204508/S288c was acquired from the Department of Microbiology, University of Ilorin. Both microscopic and cultural characterizations were performed to verify the oval and spherical budding cells (Shu *et al*., 2007). Since *Aspergillus niger* KC 329626 thrives in environments with relative humidity and storage temperatures of 33 ± 2 °C, the fungus was isolated from onion bulbs.

Isolation of Fungi from Onion Bulb: Using the serial dilution approach, fungi were separated from onion bulbs. One (1) g of the sample was diluted in 9 ml of sterile distilled water up to 10^{-5} using a serial dilution method. Using the spread plate approach, an aliquot of 0.1 ml was removed from the test tube and plated on Potato Dextrose Agar (PDA) with 0.1 ml of streptomycin. The plates were then incubated for 7 days at 28 °C (Fawole and Oso, 2004). A pure culture of the fungus was obtained by periodically subculturing it and then placed on PDA slants kept at 4 °C for later use.

Molecular Identification of Fungal Isolate: To identify the fungal isolate that was isolated from onion bulbs, the molecular analysis was evaluated. The procedure identified below was adopted. To make the buffer solution, 1 ml of dellapota buffer was weighed in 3 µl of proteinase K and the mixture was inverted. The fungi that were growing on the plate were scooped with a surgical blade, being careful not to pick the agar, and then ground in a mortar with 1 ml of extraction buffer while being stored in a 1.5 ml Eppendorf tube. After adding 50 µl of 20% SDS, the mixture was incubated for 10 mins at 60°C in a water bath then cooled to ambient temperature for 5 mins before being mixed in reverse 2 or 3 times with 100 µl of 7.5M potassium acetate. It was centrifuged for 10 mins at 13,000 rpm. A new 1.5 ml Eppendorf tube was filled with 450 µl of the clear supernatant, and 500 µl of isopropanol was added in inverse mix for2 or 3 times. The tube was centrifuged for 10 minutes at 13,000 rpm to sediment the pellet after being incubated at -20 °C for an hour and the supernatant discarded. The pellet was washed in 500 µl of 70% ethanol, and the corresponding speed was spun for 2 mins. The procedure was repeated twice, and to ensure that there was no remaining ethanol, the taps were dried and the ethanol was disposed of. The pellet was suspended in

50 µl of sterile distilled water after being dried for 15 mins in an incubator set at 37°C. It was then stored at -20°C or -80°C for future experiments (Mejic a*et al*., 2021).

Bioethanol Production by Submerged Fermentation: To ferment bioethanol, the hydrolyzed sample was placed in 250 ml cotton wool-plugged Erlenmeyer flasks, which was then autoclaved at 121 °C for 15 minutes and allowed to cool down. Each flask holding the pretreated samples was aseptically inoculated with cultures of *Aspergillus niger*, *Saccharomyces cerevisiae*, and a mixture of *Aspergillus niger* and *Saccharomyces cerevisiae*. The flasks were shaken, corked with cotton wool, and then incubated at 28 ± 2 °C for 6 days. To create a uniform solution and even dispersion of the organisms in the mixture of substrate, the flasks were shaken periodically. The samples were aseptically removed from the fermentation medium every 24 hours and centrifuged for 6 mins at 4,500 rpm. The filtrate was then used to measure the reducing sugar content, pH, total titrable acid, specific gravity, and ethanol yield using a spectrophotometer and the ethanol standard curve assay method (Singh *et al*., 2014).

Optimization Parameters for Bioethanol Production:
Effect of Incubation Time on Bioethanol Production: A pretreated sample weighing 30 g was added to Mineral Salt Medium and 100 ml distilled water which was then autoclaved and cooled. The medium was adjusted to a pH of 4.0. To inoculate the flask, a 1 ml aliquot of fungal spore suspension containing $2.0x10^{5}$ spores/ml was used. The flasks were incubated at 30 °C for 6 days at 200 rpm on a rotary shaker (Singh *et al*., 2014).

Effect of Temperature on Bioethanol Production: A pretreated sample weighing 30 g was added to Mineral Salt Medium and 100 ml distilled water which was then autoclaved and cooled. The medium was adjusted to a pH of 4.0. To inoculate the flask and 1 ml aliquot of fungal spore suspension containing $2.0x10^{5}$ spores/ml was used. The flasks were incubated for 5 days at temperatures of30 °C, 35 °C and 40 °C on a rotary shaker preset at 200 rpm (Singh *et al*., 2014).

Statistical Analysis: The Statistical Package for Social Sciences (SPSS) version 25.0 (SPSS Inc., Chicago, IL) was used to analyze all the data. The two-way analysis of variance (ANOVA) was used for comparison after the data were statistically processed to estimate the mean ± standard deviation (SD). A statistically significant result was defined as a P value of less than 0.05.

RESULTS AND DISCUSSION

Fermentation of Cassava Peels to Produce Bioethanol during Different Incubation Period: The maximum ethanol yields were obtained on day 4 for *Saccharomyces cerevisiae* (29.56 ± 0.05) and *Aspergillus niger* (26.52 ± 0.07), while the lowest yields were obtained on day 1 for *Aspergillus niger* (19.00 ± 0.50) and 20.88 ± 0.04b for *Saccharomyces cerevisiae* (Table 1).

Table 1: Fermentation of Cassava Peels to Produce Bioethanol during Different Incubation Periods

Incubation Period (Days)	Fungal Isolates	pH	TTA (mL)	Reducing Sugar (%)	Ethanol Yield (g/ml)	Specific Gravity (kg/m^3)
1	S.C	$4.60±0.05^{a}$	$0.95±0.03^{b}$	$3.56±0.34^{a}$	$20.88±0.04^{b}$	$0.98±0.05^{a}$
	A. G	$4.50±0.43^{a}$	$0.90±0.01^{b}$	$3.28±0.04^{a}$	$19.00±0.50^{b}$	$0.99±0.01^{a}$
2	S.C	$4.52±0.01^{a}$	$1.05±0.07^{a}$	$2.95±0.56^{b}$	$22.05±0.01^{a}$	$0.97±0.40^{b}$
	A. G	$4.80±0.03^{a}$	$1.07±0.60^{a}$	$2.98±0.34^{b}$	$21.01±0.06^{a}$	$0.98±0.50^{b}$
3	S.C	$4.40±0.50^{a}$	$1.20±0.45^{a}$	$2.50±0.50^{b}$	$25.98±0.50^{a}$	$0.99±0.01^{b}$
	A. G	$4.20±0.45^{b}$	$1.16±0.56^{b}$	$2.45±0.07^{a}$	$23.67±0.01^{a}$	$0.98±0.03^{a}$
4	S.C	$3.50±0.10^{b}$	$1.54±0.40^{a}$	$2.05±0.05^{a}$	$29.56±0.05^{b}$	$0.97±0.04^{a}$
	A. G	$3.85±0.56^{a}$	$1.42±0.50^{a}$	$2.12±0.01^{b}$	$26.52±0.07^{b}$	$0.98±0.05^{b}$
5	S.C	$3.53±0.01^{b}$	$1.67±0.02^{a}$	$1.86±0.65^{b}$	$25.54±0.07^{a}$	$0.99±0.01^{a}$
	A. G	$3.50±0.05^{b}$	$1.50±0.05^{a}$	$1.80±0.06^{b}$	$22.34±0.04^{b}$	$0.97±0.02^{b}$

The values are the average of three readings plus the standard deviation. P < 0.05 indicates a significant difference between values on the same column with different Superscrip; Key: S.C – *Saccharomyces cerevisiae*; AG - *Aspergillus niger*

The production of bioethanol is gaining a lot of attention globally and has been extensively researched because of its tremendous significance and application as a type of biofuel. Cassava peels were used as the substrate for this study because it was recently noted that lignocelluloses and celluloses are the primary biomass source for the manufacture of bioethanol (Sun and Cheng, 2011). As a cheap, renewable, and abundant source of energy, cassava peels are valuable resources (Chohan *et al*., 2020: Singh *et al*., 2014). Due to the constant production of these agro-wastes from farms and other places where it is being used, it builds up annually in significant amounts and causes environmental issues. But among other things, they could be used to produce

bioethanol and enzymes (Al-Dulaimi*et al*., 2018: Tanino *et al*., 2012).

pH During Fermentation of Cassava Peels to Produce Bioethanol at Different Temperatures: The pH value recorded by *Saccharomyces cerevisiae* at various temperatures ranged from 3.00±0.01a to 4.20±0.05b, with day 1 having the greatest pH value at 35°C and day 6 having the lowest. *Aspergillus niger* produced a pH value that varied from 3.10±0.05b to 4.5±0.05a, with day 1 having the maximum temperature of 35°C and Day 6 having the lowest temperature of 40°C (Figure 1–3).

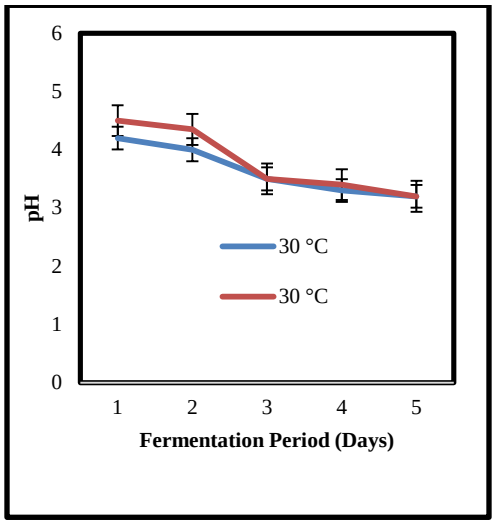

Fig. 1: pH during Fermentation to Produce Bioethanol at 30 °C

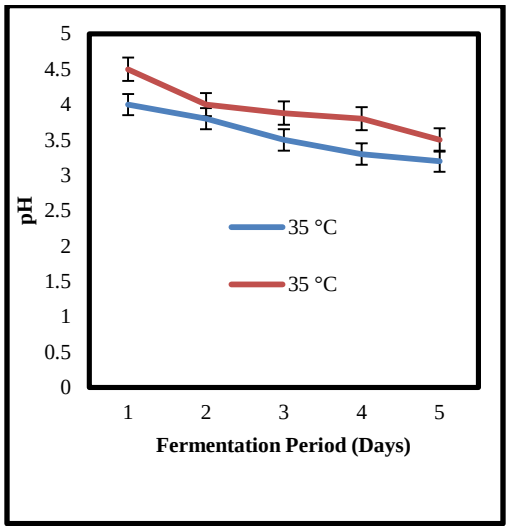

Fig. 2: pH during Fermentation to Produce Bioethanol at 35 °C

Throughout the five days of fermentation period, the pH varied. The fermenting broth becomes more acidic as the pH reduced, altering the metabolic processes of the organism to produce more ethanol. However, ethanol production was increased and bacterial contaminants were deterred by the acidic pH seen during fermentation (Oyeleke and Jubrin, 2019). This result is consistent with Nester *et al*. (2001). The synthesis of bioethanol also significantly increased when the pH decreased.

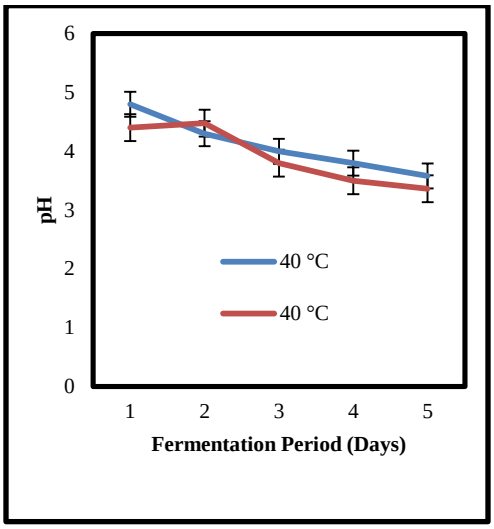

Fig. 3: pH during Fermentation to Produce Bioethanol at 40 °C

Total Titrable Acid (TTA) during Fermentation of Cassava Peels to Produce Bioethanol at Different Temperatures: At varying temperatures, *Saccharomyces cerevisiae* produced a total titrable acid value ranging from 0.60±0.01 to 1.21±0.07, with day 6 peaking at 35°C and day 1 lowering to 30°C. Using *Aspergillus niger*, the total titrable acid value varied between 0.62±0.05 and 1.25±0.02, with day 6 yielding the highest temperature of 35°C and day 1 yielding the lowest temperature of 30°C (Fig. 4–6).

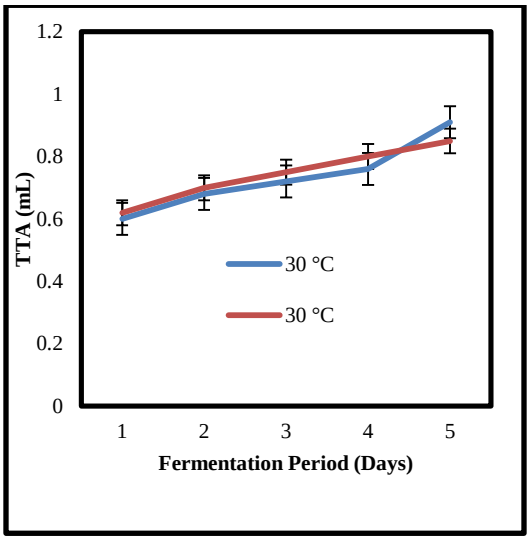

Fig. 4: TTA during Fermentation of Cassava Peels to Produce Bioethanol at 30 °C

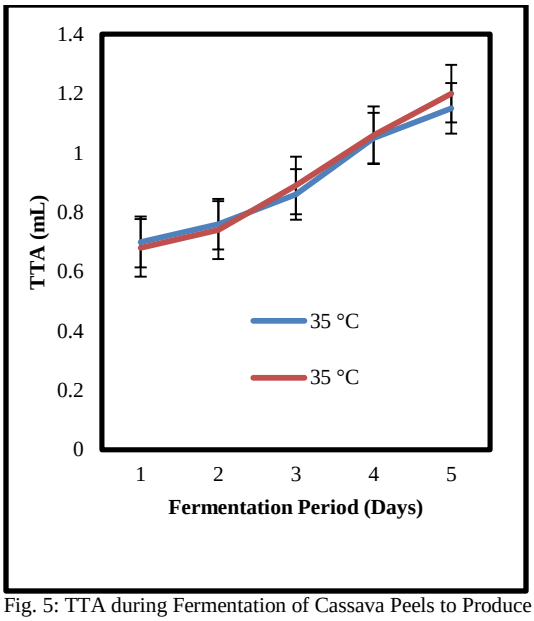

Fig. 5: TTA during Fermentation of Cassava Peels to Produce Bioethanol at 35 °C

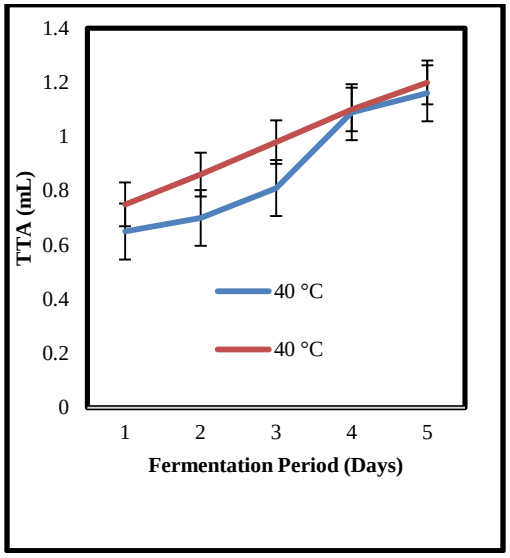

Fig. 6: TTA during Fermentation of Cassava Peels to Produce Bioethanol at 40 °C

Reducing Sugar during Fermentation of Cassava Peels to Produce Bioethanol at Different Temperatures: The reducing sugar value recorded by *Saccharomyces cerevisiae* at varying temperatures ranged from 1.65±0.01 to 3.20±0.05, with day 1 recording the highest temperature of 40°C and day 6 recording the lowest temperature of 35°C. At a temperature of 30°C, the maximum values was observed at day 1 and the lowest at day 6, the reducing sugar value recorded by *Aspergillus niger* varied from 1.76±0.05 to 3.15±0.03 (Figures 7–9). The use of free sugars by filamentous fungus and yeast may have contributed to the rise in total titratable acidity (Akinyele, 2014). However, the results did not demonstrate a direct correlation between TTA and bioethanol yield, which can be explained by the ability of the fungi to synthesize other metabolites (Rajkovic et al., 2007). This is in relation to the study conducted by Braide et al. (2016) who reported that TTA increased in respect to substrate concentration. *Saccharomyces cerevisiae* fermentation of the substrates demonstrated that the yield of bioethanol was proportional to the fermentation period until a drop occurred after 4 days, at which point the yield increased as the fermentation period increased. This correlation, which is consistent with findings of Chen et al. (2010), resulted from utilization of the sugar by the yeast.

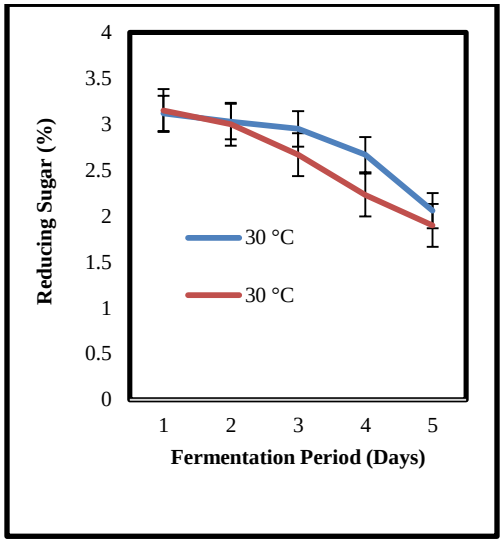

Fig. 7: Reducing Sugar during Fermentation of Cassava Peels to Produce Bioethanol at 30 °C

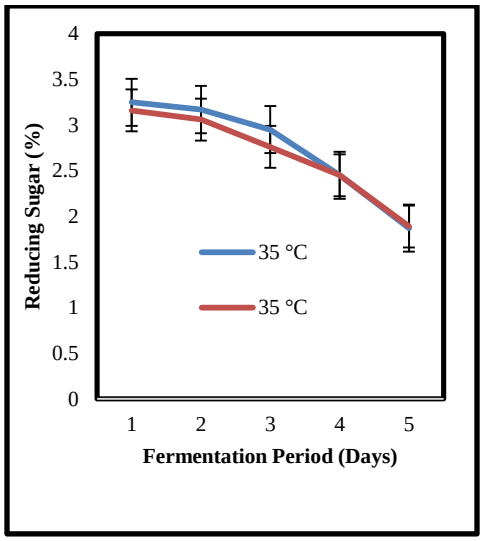

Fig. 8: Reducing Sugar during Fermentation of Cassava Peels to Produce Bioethanol at 35 °C

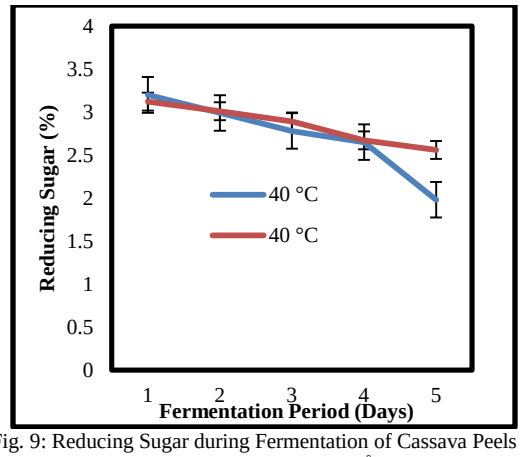

Fig. 9: Reducing Sugar during Fermentation of Cassava Peels to Produce Bioethanol at 40 °C

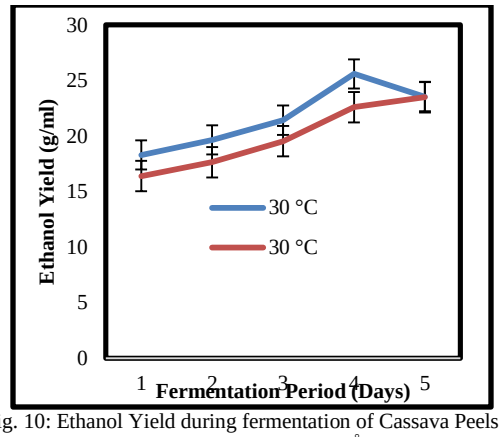

Fig. 10: Ethanol Yield during fermentation of Cassava Peels to Produce Bioethanol at 30 °C

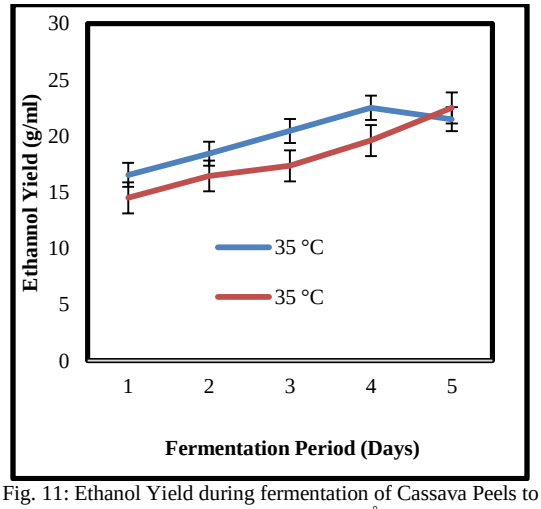

Fig. 11: Ethanol Yield during fermentation of Cassava Peels to Produce Bioethanol at 35 °C

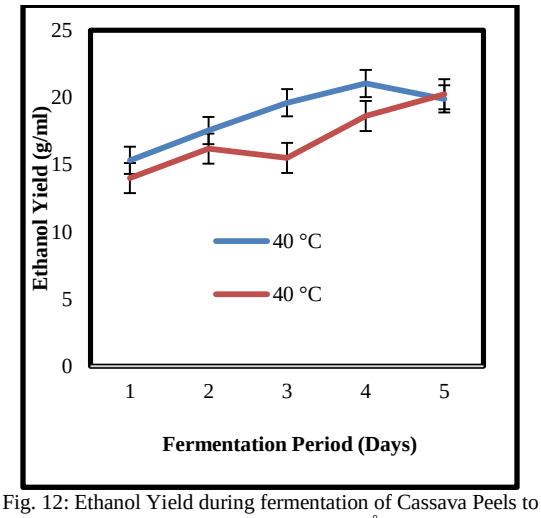

Fig. 12: Ethanol Yield during fermentation of Cassava Peels to Produce Bioethanol at 40 °C

As the fermentation duration increased, it was found that the amount of sugar left in the fermentation media decreased. According to Braide *et al*. (2016), this may be explained due to the use of sugar by microorganisms as a carbon source for growth, energy, and metabolic processes, which in turn leads to the synthesis of ethanol. The yeast directly turned these reducing sugars into alcohol; the amount and rate of alcohol production are determined by the concentration of these reducing sugars. The reason for the decline is that the yeast needs the sugars for energy and, in turn, to make ethanol. The reducing sugar that was obtained is nearly similar to what Subramanian *et al*. (2010) reported.

Bioethanol Yield during Fermentation of Cassava Peels to Produce Bioethanol at Different Temperatures: With *Saccharomyces cerevisiae*, the bioethanol yield value at various temperatures varied from 15.32±0.50 to 25.6±0.62, with Day 4 having the maximum temperature of 30°C and Day 1 having the lowest at 40°C. *A. niger* produced bioethanol with a yield value ranging from 14.00±0.04 to 23.54±0.03, with the highest temperature at 30°C occurring on Day 4 and the lowest temperature at 40°C on Day 1 (Figure 10-12).

When producing bioethanol, the incubation temperature is essential. At fermentation temperatures between 30 and 35 °C, the output increased slightly; however, as fermentation temperatures increased, the yield dropped off drastically.

This is comparable to the findings of ElGazzar and Ismail (2020), who found that *S. cerevisiae* produced the most of bioethanol at 30 °C. The output of bioethanol at different temperatures showed that the best incubation temperature for bioethanol production was at 30 °C for fermentation.

The percentage of bioethanol produced decreased as the temperature increased further. The *Saccharomyces cerevisiae* fermentation of the substrates demonstrated that the production of

bioethanol increased with an increase in fermentation time, but it was proportional to the fermentation period until a decline occurred after 4 days. This correlation, which is consistent with findings of Chen *et al*. (2010) results from ongoing use of the sugar by yeast. Significant differences were found in the amount of bioethanol produced by the examined fungi during the course of the incubation periods ($P<0.05$). It was noted that the production of bioethanol increased during the incubation periods until day four, after which it fell once more until the fifth day. Significant differences were recorded in the amount of bioethanol produced by the identified fungi during the course of the incubation period ($P<0.05$). It was noted that the production of bioethanol increased during the incubation period until day 4, after which it dropped once more until the fifth day. Cell activity increased with longer incubation period, lowering sugar levels quickly, and producing more alcohol. The same pattern was observed in the decline of the specific gravity and decreased sugar content.

Specific Gravity during Fermentation of Cassava Peels to Produce Bioethanol at Different Temperature: Aspergillus niger and *Saccharomyces cerevisiae* at several temperatures recorded specific gravities ranging from 0.97±0.01 to 0.99±0.02. At day 2, *Aspergillus niger* had the lowest temperature at 30 °C, whereas at day 3, *Saccharomyces cerevisiae* had the highest temperature at 35 °C (Table 2).

Table 2: Specific Gravity during Fermentation of Cassava Peels to Produce Bioethanol at Different Temperature

		Fermentation Days				
Temp (^{0}C)	Fungal Isolates	**1**	**2**	**3**	**4**	**5**
30	S. C	0.99±0.05^a	0.98±0.05^a	0.96±0.01^a	0.99±0.01^a	0.98±0.01^a
	A. G	0.98±0.01^b	0.97±0.05^b	0.99±0.01^b	0.98±0.05^b	0.99±0.01^b
35	S. C	0.98±0.01^a	0.99±0.01^a	0.97±0.05^b	0.98±0.05^a	0.99±0.05a
	A. G	0.98±0.05^a	0.99±0.01^a	0.99±0.00^a	0.98±0.01^a	0.97±0.05^b
40	S. C	0.99±0.00^a	0.98±0.01^a	0.97±0.00^a	0.98±0.01^a	0.99±0.01^b
	A. G	0.99±0.00^a	0.98±0.00^a	0.97±0.00a	0.99±0.00^b	0.98±0.00^a

Values are means of triplicate reading ± Standard deviation of specific gravity of cassava peels at different temperatures. Values on the same column with different superscripts are significantly different at $P < 0.05$.

Key: S.C – *Saccharomyces cerevisiae*; AG - *Aspergillus niger:* What resulted into the drop in specific gravity was the fermentation of the entire soluble material into bioethanol. This finding is consistent with that of Aisien *et al*. (2010), who found that during the fermenting periods, the sugar content and specific gravity of agro-waste dropped, respectively. Cell activity increased as the incubation period is extended, lowering decreasing sugar levels, which likewise increased quickly, and producing more alcohol. Since over 96% of the starchy component of the peel was converted to simple reducing sugar during the submerged fermentation, the amylolytic character of cassava peel may be the reason for its effective breakdown by *A. niger* and *S. cerevisiae*. This observation aligns with the previous research conducted by Omemu *et al*. (2015) and Okolo *et al*. (2017). Similar outcomes have also been reported in cases where cassava peel was degraded using *Rhizopus* and *Trichoderma* species (Obadina*et al*., 2006). It was observed that the specific gravity increased as the acid concentration increased, this is accordance with the studies conducted by Marx and Nquma (2013) and Ado et al. (2009) on cassava starch. The findings of Jimoh et al. (2009) and Ajay et al. (2014), who reported that ethanol yield increased as substrate concentration increased, were also supported by our investigation. The specific gravity reduced during fermentation, and the sugar content decreased noticeably as well. Since the sugar in the broth fermented to alcohol, the decrease in total soluble solids may be the cause of the decrease in specific gravity (Braide et al., 2016). Cell activity rises with an extended incubation period, lowering the sugar levels while producing more alcohol at a faster rate. The pattern of depletion in specific gravity and lowering sugar content was similar. The study also showed that, in comparison to *A. niger*, *S. cerevisiae* produced more reducing sugar while hydrolyzing the cassava peel. As the fermentation process continued, the amount of ethanol produced increased due to the extremely quick depletion of sugar. Active fermentation may be a sign of the exponential phase, which is linked to fast cell division (Amerine, 2000). It was revealed that the combination of *A. niger* and *S. cerevisiae* gave considerably higher ethanol yield. This was more than what Chohan *et al*. (2020), who used cassava peels to make bioethanol, reported. This might be because the two species utilized in this study, *A. niger* and *S. cerevisiae*, work in concert. However, compared to *A. niger*, *S. cerevisiae* produced more bioethanol. According to previously published findings, the fermentation duration had a

significant impact on the amount of bioethanol that yeasts produced (Enan *et al*., 2018). After two days of growth, it was determined that employing actively developing yeast cells in the early to mid-exponential phase of growth, yeasts produced the highest amounts of bioethanol through fermentation of sugary substrates (El-Sayed *et al*., 2015). The moderate drop in specific gravity was caused by the fermentation of the whole soluble material into bioethanol. This observation is in line with the results of Aisien et al. (2010), who reported that during the fermentation period, the specific gravity and sugar content of the agro-wastes decreased, respectively.

For the formation of bioethanol, the ideal temperature, incubation time, and substrate concentration were 30 °C, 4 days, and 30 g/100 ml for both species. These findings go in contrast to the research of Yan *et al*. (2020), who discovered that 25 °C was the ideal temperature for producing bioethanol since *S. cerevisiae* (ATCC 204508/S288c) can create bioethanol at higher temperatures and is therefore more suited for industrial bioethanol production. The optimization of fermentation conditions for increased bioethanol yields by certain yeasts was investigated (Hashem *et al*., 2021). They discovered that the incubation temperature had an impact on the synthesis of bioethanol; the ideal incubation temperature seems to be between 30 and 35 °C. The mesophilic nature of the yeast serves as evidence for this study.

Conclusion: Cassava peels, an agricultural waste, can be used to make bioethanol, according to the findings of this study. Although it is possible to produce bioethanol from cassava peels, a number of variables, particularly temperature, pH, duration, and substrate concentration, might affect the yield. The co-culture of *Aspergillus niger* KC 329626 and *Saccharomyces cerevisiae* ATCC 204508/S288c was used in a fermentation method that produced the highest ethanol production. *S. cerevisiae* and *A. niger* may be the best combination for producing bioethanol from cassava peels. Because of its low cost and ability to reduce pollution in the environment, using cassava peels to produce bioethanol is a worthy alternative. This makes the process of producing bioethanol cost-effective, environmentally benign, and renewable.

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Data Availability statement: data are available upon request from the corresponding author

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