



STUDY ON THE EFFECT OF TEMPERATURE ON BIO-ETHANOL PRODUCTION FROM CASSAVA FLOUR AND CASSAVA PEELS: AN INSIGHT INTO BIO-ENERGY PROCESSES

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ABSTRACT

Knowledge of fermentation parameters with respect to temperature is necessary in bio-ethanol production; as an important process in bio-energy applications for green energy utilization. Bio-ethanol provides an alternative clean energy source that can be obtained from biomass, thereby mitigating pollution problems associated with using environmentally unfriendly energy sources. A study into the effect of temperature on bio-ethanol production from cassava flour and cassava peels was investigated. Temperature was varied between 30-60°C at 5°C intervals and the volume of bio-ethanol produced was examined using optical density measurement. An increase in substrate concentration led to a proportionate increase in the volume of bio-ethanol produced at an optimal temperature of 30°C. However, a gradual decrease in bio-ethanol production was observed beyond 30°C (35-60°C), which shows the effect of temperature on bio-ethanol production from cassava flour and cassava peels, with the yeast activity optimum at 30°C using 80 grams of substrate. The optical density measurements provided a reliable indication of optimum microbial activity and bio-ethanol production. Bio-ethanol yield was higher in cassava flour than in cassava peels at the same concentration, indicating higher carbohydrate content in cassava flour. The findings show a significant temperature influence on the activity of the yeast efficiency in bio-ethanol production. Cassava and its peel are important for the production of bio-ethanol because it holds potential as a valuable feedstock for bio-ethanol production, offering a sustainable solution to waste management and clean energy; therefore, knowledge of the optimal fermentation temperature is an important information in bio-ethanol production from cassava precursors.

Keywords: Bio-ethanol, Cassava flour, Cassava peel, Optical density, Temperature, Yeast

INTRODUCTION

Fossil energy sources such as natural gas, coal, petroleum, and oil, used for the production of fuel, electricity, and petrochemicals are gradually depleting due to the increase in human population and industrial activity. The utilization of these conventional fossil energy sources in the long run causes the depletion of energy sources which is not sustainable (Gallezot, 2019). Fossil fuel which is a non-renewable energy source has considerable negative environmental impact due to the generation of green house gases, causing climate distortion and changes. The global pursuit of sustainable energy sources has intensified in response to the challenges posed by climate change and dwindling fossil fuel reserves (Singh & Kumar 2017). Among renewable energy alternatives, bio-ethanol has emerged as a promising option due to its potential to mitigate greenhouse gas emissions and enhance energy security. Bio-energy from renewable resources becomes an alternative replacing fossil fuels. The raw materials for alternative renewable bio-energy sources are available and abundant. Almost all petroleum-based fuels can be replaced by renewable fuels produced from biomass such as bio-ethanol, bio-diesel, bio-hydrogen, etc. The demand for bio-ethanol as a widely utilized bio-fuel has been increasing, necessitating the need to enhance its production from environmentally friendly, affordable and readily available precursor materials (Muhammad *et al.*, 2023). Bio-ethanol, an ideal bio-energy source has some advantages such as high-octane fuel and reduced pollution emission (Hahn-Hägerdal *et al.*, 2006). It is a clean and renewable bio-fuel with major environmental benefits such as the burning of oxygenated fuel mixture consisting of ethanol and gasoline, reducing pollution emissions. In response to the overwhelming global energy requirement, depletion in fossil

resources and high cost of petroleum-based fuels, researchers are actively seeking alternative sources of energy, with a focus on bio-ethanol from renewable sources (Muhammad *et al.*, 2023). Bio-ethanol obtained from agricultural materials and agro-wastes offers a sustainable alternative to conventional energy sources, such as fossil fuels, as the demand for energy continues to increase and the supply depletes (Lawan *et al.*, 2023). In Malaysia for example, agricultural wastes are major feedstock for producing bio-ethanol due to low cost and ready availability (Lawan *et al.*, 2023). The utilization of agricultural waste for bio-ethanol production also does not disturb the consumer food supply as it is based on waste-to-energy concept (Lawan *et al.*, 2023). Understanding the multifaceted importance of cassava lays the foundation for exploring its potential as a feedstock for bio-ethanol production in Nigeria, because of the country's large cassava production capacity and its huge availability. Cassava botanically known as *Manihot esculenta crantz*, is a vital crop in the agricultural landscape of many tropical and subtropical regions, serving as a primary source of food, feed, and industrial raw material. Its significance stems from its adaptability to diverse agro-ecological conditions, resilience to pests and diseases, and high productivity under low-input agricultural systems (FAO, 2017). Cassava plays a pivotal role in ensuring food security, income generation, and poverty alleviation, particularly in sub-Saharan Africa, Asia, and Latin America, where it serves as a dietary staple for millions of people (Ceballos *et al.*, 2017). Cassava processing generates substantial quantities of waste, including cassava peels, which if not properly managed, can lead to environmental problems (Adeniyi & Adeeyo, 2020). Cassava peel holds potential as a valuable feedstock for bio-ethanol production, offering a sustainable solution to waste

management, while simultaneously contributing to the renewable energy sector. Cassava roots are rich in starch, making them a valuable source of dietary carbohydrates for human consumption. The roots can be processed into various food products such as flour, starch, and snacks, offering versatility in culinary applications and dietary diversity (Nweke *et al.*, 2022). Cassava leaves, though less commonly consumed, is also nutritious and serves as a source of protein, vitamins, and minerals in some regions. The crop's ability to provide sustenance and nutritional security to populations in resource-constrained environments underscores its importance in combating hunger and malnutrition. In addition to the role of cassava as a food crop, cassava holds significant potential for industrial bio-energy applications, particularly in the production of bio-ethanol, a renewable fuel with promising environmental and economic benefits, having the unique advantage of reducing CO₂ emissions by up to 18% (Fathima *et al.*, 2023; Ridwan *et al.*, 2023). The bio-ethanol industry offers opportunities for rural development, job creation, and value addition along the cassava value chain, contributing to economic growth and poverty alleviation in cassava-producing regions ((FAO, 2017). By harnessing cassava's carbohydrate-rich biomass for bio-ethanol production, countries can reduce their dependence on fossil fuels, mitigate greenhouse gas emissions, and promote sustainable energy solutions. Temperature exerts a profound influence on microbial fermentation kinetics and product formation during bio-ethanol production. Most fermentative microorganisms exhibit temperature-dependent growth and metabolic activity, with optimal fermentation temperatures varying among different strains. *Saccharomyces cerevisiae*, commonly used in bio-ethanol production for example, typically performs optimally at temperatures between 30°C and 40°C (Ekwe *et al.*, 2024). Deviations from the optimal temperature range can alter microbial growth rates, substrate utilization patterns, and fermentation by-product profiles, affecting ethanol yield and productivity. Furthermore, temperature fluctuations during fermentation can induce cellular stress responses and compromise yeast viability, leading to reduced fermentation performance. Research has demonstrated the feasibility of bio-ethanol production from cassava and its by-products (Chantima *et al.*, 2021; Fathima *et al.*, 2023; Ridwan *et al.*, 2023; Amaoma *et al.*, 2025). However, optimizing fermentation parameters, particularly temperature, remains a critical area of investigation. Temperature plays a pivotal role in influencing microbial activity, enzyme kinetics, and biochemical reactions during the fermentation process. Despite its importance, the optimal temperature range for bio-ethanol production from cassava flour and cassava peel has not been conclusively determined. Thus, there is a pressing need for systematic research to elucidate the effect of temperature on bio-ethanol yield, aiming to optimize production processes and enhance the sustainability of bio-ethanol production from cassava flour and cassava peel.

Therefore, investigating the effect of temperature on the fermentation of cassava flour and cassava peels to ascertain the optimal value constitutes an important focus in the production of bio-ethanol from cassava precursors in this study.

MATERIALS AND METHODS

Materials

Beakers, burette, clamp, retort stand, flasks, fermentation vessels, measuring cylinder, pH meter, rubber tubes, spatula, conical flasks, test tubes, refrigerator, stirring rod, thermometer, weighing balance, grinder, thermostated water bath, oven, cassava tubers, aluminium foil, yeast (*Saccharomyces cerevisiae*), ethanol, autoclave, UV Spectrophotometer (JENWAY 6703).

Cassava Substrate Sourcing and Preparation

Cassava tubers were sourced from Glorious Vision University farm, Ogwa, in Esan West Local Government Area of Edo State, South-South Nigeria. The University is bounded by the geographical coordinates of 6°30'57"N and 6°11'46"E. Sourced cassava tubers were washed to remove soil and debris, peeled, and chopped into smaller pieces of approximately 2 cm³. The chopped tubers were air-dried in open air for 24 hours, after which they were grinded into fine powdery cassava flour; while the peels were thinly sliced. This was to increase the surface area for enzymatic action.

Experimental Procedure

Cassava flour and its peels were fermented at varying substrate masses of between 20 g to 80 g in a fermentation vessel containing 2 g of yeast enzyme. Temperature was varied between 30-60°C for each of the fermentation vessels using a thermostated water bath. Ethanol is oxidized to acetic acid with an excess of potassium dichromate in the presence of sulphuric acid, giving off a blue-green color. This indicates the conversion of the carbon source to bio-ethanol which is a measure for its quantitative determination. The amount of bio-ethanol produced was quantified by developing a standard curve with pure ethanol and a series of dilutions made from Jones reagent (0.5 mL of concentrated H₂SO₄ and 1 mL of 2% K₂Cr₂O₇). The mixture (Jones reagent) was then added to 0.5 mL of absolute ethanol, and 3.5 mL of distilled water was added. This was repeated for 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mL of absolute ethanol, and their optical density was determined at a wavelength of 600 nm using a UV spectrophotometer (JENWAY 6703) for the various ethanol concentrations. The optical density for each of the transmittance of the amount of ethanol was calculated using the equation $OD = -\log_{10}(T) = \log_{10}(I_0 / I)$.

RESULTS AND DISCUSSION

The results showing variation in volume of bio-ethanol produced from the different substrates with respect to temperature are presented in the Tables 1-4 and Figures 1-8.

Table 1: Amount of bio-ethanol produced from cassava flour (20 g and 40 g) at varied temperatures (2 g yeast, 2 mL of 2% K₂Cr₂O₇, and 1 mL H₂SO₄)

Cassava flour (20 g)			Cassava flour (40 g)		
Temp. (°C)	Optical Density (600 nm)	Bio-ethanol (mL)	Temp. (°C)	Optical Density (600 nm)	Bio-ethanol (mL)
30	0.034	3.810	30	0.049	5.410
35	0.031	3.590	35	0.044	5.320
40	0.025	3.140	40	0.041	3.220
45	0.020	1.460	45	0.038	2.990
50	0.018	1.120	50	0.029	2.760
55	0.016	0.450	55	0.027	1.220
60	0.015	0.320	60	0.022	1.010

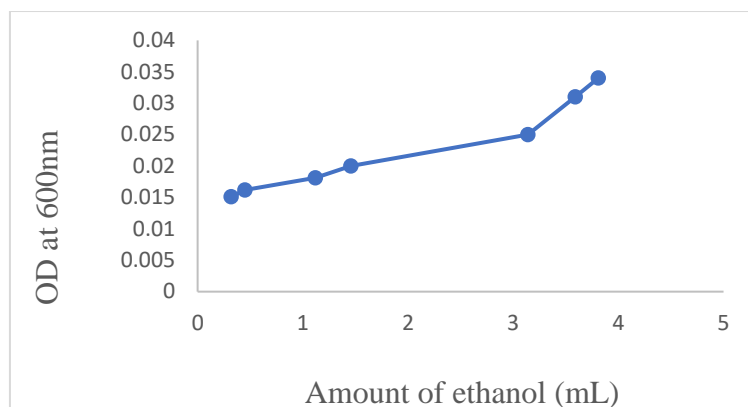


Figure 1: Graphical profile of optical density against bio-ethanol produced (20 g of cassava flour)

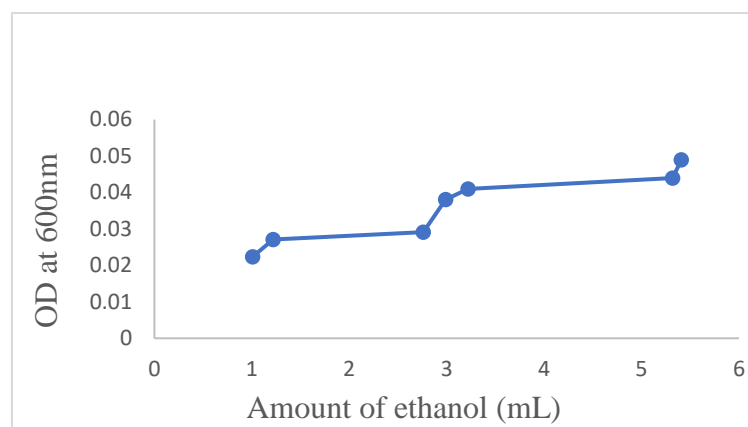


Figure 2: Graphical profile of optical density against bio-ethanol produced (40 g of cassava flour)

Table 2: Amount of bio-ethanol produced from cassava flour (60 g and 80 g) at varied temperatures (2 g yeast, 2 mL of 2% $K_2Cr_2O_7$, and 1 mL H_2SO_4)

Cassava flour (60 g)			Cassava flour (80 g)		
Temp (°C)	Optical Density (600 nm)	Bio-ethanol (mL)	Temp. (°C)	Optical Density (600 nm)	Bio-ethanol (mL)
30	0.050	5.460	30	0.061	5.500
35	0.045	5.420	35	0.052	5.440
40	0.040	3.200	40	0.049	5.410
45	0.038	2.970	45	0.044	3.990
50	0.028	2.740	50	0.032	3.720
55	0.027	1.250	55	0.028	2.810
60	0.023	1.040	60	0.025	1.230

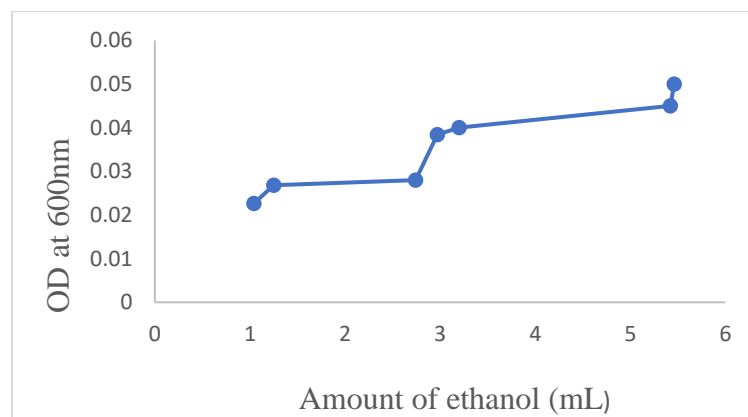


Figure 3: Graphical profile of optical density against bio-ethanol produced (60 g of cassava flour)

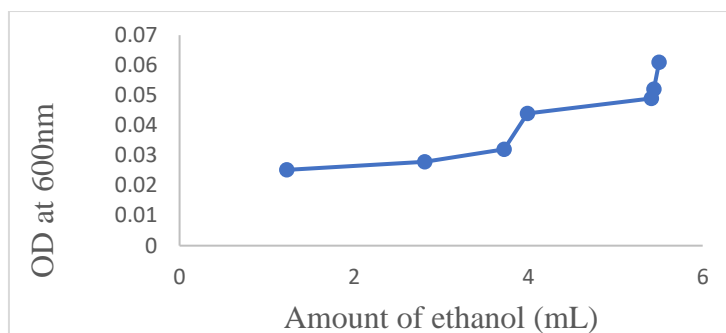


Figure 4: Graphical profile of optical density against bio-ethanol produced (80 g of cassava flour)

Table 3: Amount of bio-ethanol produced from cassava peels (20 g and 40 g) at varied temperatures (2 g yeast, 2 mL of 2% $K_2Cr_2O_7$, and 1 mL H_2SO_4)

Cassava peels (20 g)			Cassava peels (40 g)		
Temp. (°C)	Optical Density (600nm)	Bio-ethanol (mL)	Temp. (°C)	Optical Density (600nm)	Bio-ethanol (mL)
30	0.028	3.310	30	0.028	3.330
35	0.026	2.910	35	0.026	2.930
40	0.024	2.780	40	0.024	2.800
45	0.019	1.310	45	0.019	1.340
50	0.017	1.020	50	0.017	1.040
55	0.015	0.390	55	0.015	0.410
60	0.015	0.291	60	0.015	0.290

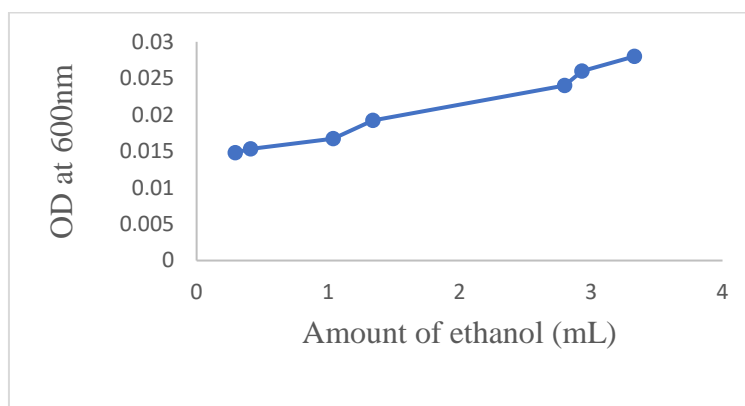


Figure 5: Graphical profile of optical density against bio-ethanol produced (20 g of cassava peels)

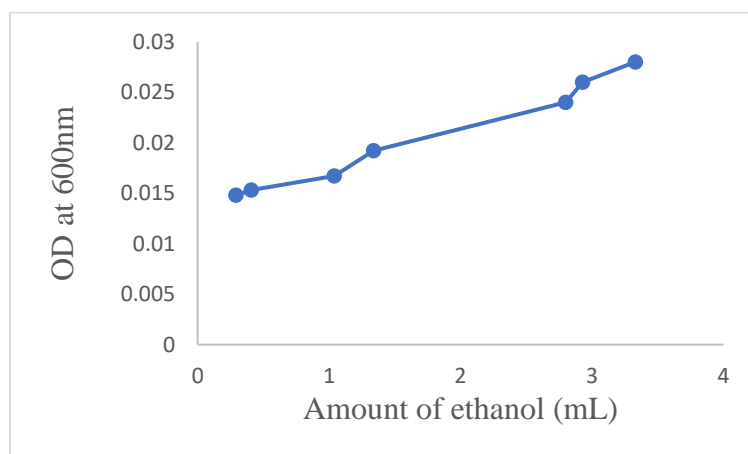


Figure 6: Graphical profile of optical density against bio-ethanol produced (40 g of cassava peels)

Table 4: Amount of bio-ethanol produced from cassava peels (60 g and 80 g) at varied temperatures (2 g yeast, 2 mL of 2% K₂Cr₂O₇, and 1 mL H₂SO₄)

Cassava peels (60 g)			Cassava peels (80 g)		
Temp. (°C)	Optical Density (600 nm)	Bio-ethanol (mL)	Temp. (°C)	Optical Density (600 nm)	Bio-ethanol (mL)
30	0.030	3.350	30	0.032	3.370
35	0.028	2.950	35	0.030	2.970
40	0.026	2.820	40	0.028	2.840
45	0.019	1.360	45	0.020	1.380
50	0.017	1.060	50	0.017	1.080
55	0.016	0.430	55	0.016	0.450
60	0.015	0.300	60	0.015	0.390

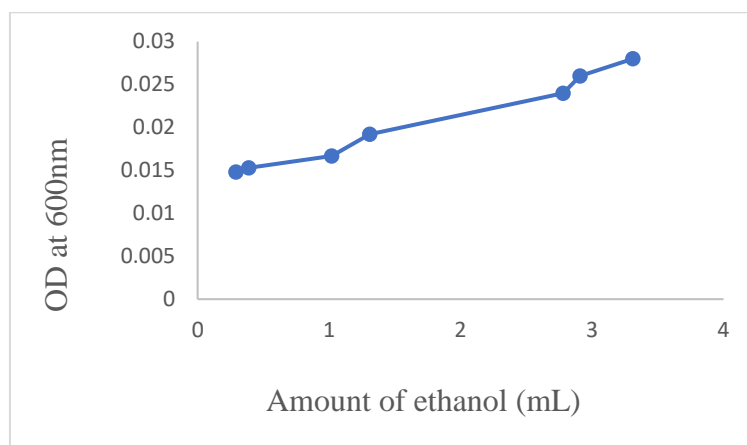


Figure 7: Graphical profile of optical density against bio-ethanol produced (60 g of cassava peels)

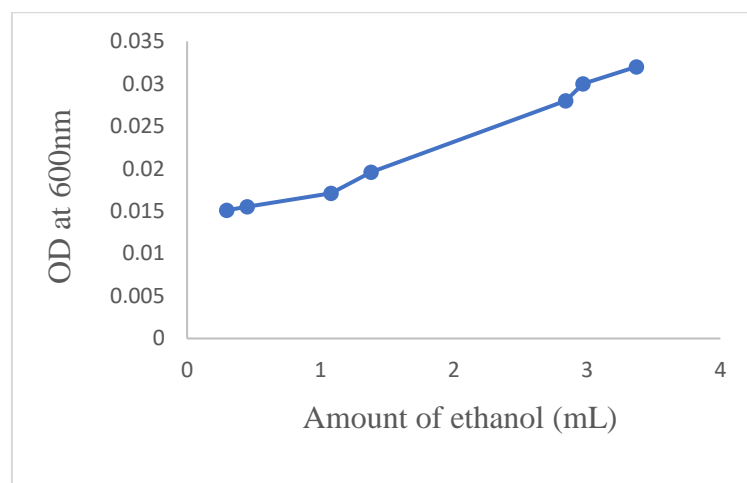


Figure 8: Graphical profile of optical density against bio-ethanol produced (80 g of cassava peels)

The effect of temperature on bio-ethanol production from cassava flour and cassava peels at substrate concentration ranges of 20-80 g is presented in Tables 1-4.

The highest volume of bio-ethanol production was recorded at 30°C, with a steady decline as temperatures increased to 60°C for both substrate (cassava flour and cassava peel) concentrations (Table 1-4). The temperature of 30°C is therefore observed to be the optimal temperature for bio-ethanol production from cassava flour.

This trend aligns with findings from Ekwe et al. (2024), who noted optimal yeast activity and bio-ethanol yields at temperatures between 30°C and 40°C for *Saccharomyces cerevisiae*. It also agrees with optimal temperature of 32.5°C reported for the production of bio-ethanol from Gamba grass

and Love grass using *Saccharomyces cerevisiae* (Muhammad et al., 2023). As also seen in the findings of Muhammad et al. (2023), bio-ethanol yield was observed to increase with increasing temperature up to 32.5°C, but showed a decline in bio-ethanol yield as fermentation temperature increased beyond for the production of bio-ethanol from Gamba grass and Love grass using *Saccharomyces cerevisiae*; thus corroborating the observed trend in this study.

The observed decrease in ethanol production at higher temperatures likely results from enzyme denaturation and inhibited microbial activity at increased temperatures. This pattern underscores the importance of maintaining moderate temperatures to preserve enzyme structure and yeast viability during fermentation. The data confirms that a low

fermentation temperature of 30°C not only maximizes yield, but may also reduce the energy cost of cooling systems, providing an advantage in industrial applications. Studies by Liszkowska and Berłowska (2021) highlight similar energy efficiencies gained by operating within yeast's optimal temperature range. Furthermore, the data underscore the environmental and economic benefits of utilizing cassava peels, supporting the findings of Chantima *et al.* (2021) and Amaoma *et al.* (2025) that bio-ethanol production from cassava by-products and its wastes offers a sustainable waste-to-energy solution.

Optical density readings (600 nm) as presented in Tables 1–4 and represented in Fig. 1-8 show a correlation between

ethanol concentration and microbial activity, with maximum peak optical density observed at 30°C, where bio-ethanol yield was highest. The optical density measurements provided a reliable indication of microbial activity and bio-ethanol production. This approach for estimating ethanol concentration aligns with methodologies recommended by Singh & Kumar (2017) for monitoring fermentation efficiency. The consistent reduction in optical density at temperatures above 35°C as observed in all substrate concentrations (Fig. 1–8,) suggests diminished yeast metabolic activity, supporting the hypothesis that enzyme stability is compromised under elevated temperatures.

Effect of substrate concentration on bio-ethanol production

Table 5: Bio-ethanol produced from different substrate (Cassava flour) concentrations at various temperatures

Substrate (g/mol)	Temperature (°C)						
	Volume of ethanol produced (mL)						
30	30	35	40	45	50	55	60
20	3.81	3.59	3.14	1.46	1.12	0.45	0.32
40	5.41	5.32	3.22	2.99	2.76	1.22	1.01
60	5.46	5.42	3.20	2.97	2.74	1.25	1.04
80	5.50	5.44	5.41	3.99	3.72	2.28	1.23

Table 6: Bio-ethanol produced from different substrate (Cassava peels) concentrations at various temperatures

Substrate (g/mol)	Temperature (°C)						
	Volume of ethanol produced (mL)						
30	30	35	40	45	50	55	60
20	3.31	2.91	2.78	1.31	1.02	0.39	0.29
40	3.33	2.93	2.80	1.34	1.04	0.41	0.29
60	3.35	2.95	2.82	1.36	1.06	0.43	0.29
80	3.37	2.97	2.84	1.38	1.08	0.45	0.29

The data in Tables 5 and 6 highlight the effect of substrate concentration on the efficiency of enzymatic production of bio-ethanol from cassava flour and cassava peels respectively. The results show that higher substrate concentrations increase bio-ethanol production yield at optimal temperatures (30°C). It is observed that as the substrate concentration increased, there was an increase in the volume of bio-ethanol produced at 30°C. But as the temperature increased in the range of 35°C to 60°C, the volume of bio-ethanol produced gradually decreased. This shows that the production of bio-ethanol from cassava flour and cassava peels was gradually affected by an increase in temperature even at increased substrate concentration, where the activity of yeast was optimum only at 30°C. Higher substrate availability enhances microbial metabolism. However, even at optimal substrate concentrations (80 g) for both substrates, ethanol yields still decrease as temperatures exceed 35°C; as seen in Tables 5 and 6. This finding correlates with studies by Hahn-Hägerdal *et al.* (2006), which reported that elevated substrate availability supports initial enzyme activity, but is offset by temperature-sensitive declines in microbial efficiency. It is seen from the results that while substrate concentration enhances production, temperature control remains critical.

A comparative assessment of the yield of bio-ethanol from both substrates using data presented in Tables 5–6 shows that cassava flour gave more bio-ethanol yield than cassava peels under identical conditions. This may be due to the lower starch content in peels compared to the flour, affecting the overall fermentable sugar availability for yeast metabolism. This aligns with the findings of Jayaraman *et al.* (2017) and Busic *et al.* (2018), where they reported that substrates with higher carbohydrate content and fermentable sugar

availability yield more bio-ethanol. Despite this, the use of cassava peels offers an environmentally beneficial approach to bio-ethanol production, aligning with Adeniyi and Adeeyo's (2020) advocacy for sustainable bio-fuel production from agricultural waste.

CONCLUSION

The findings from this study successfully established the optimal fermentation temperature for the production of bio-ethanol from cassava flour and cassava peels. Maintaining this temperature during bio-ethanol production from cassava precursors is seen to be an energy-efficient approach compared to other fermentation processes that utilize higher temperatures, which will require additional cooling mechanisms. Cassava flour and its peel therefore hold potential as a valuable feedstock for cost-effective bio-ethanol production at minimal operating temperatures, offering a sustainable solution to waste management, energy conservation, and clean energy. Knowledge of the optimal fermentation temperature is an important information in bio-ethanol production from cassava precursors, which is also essential for enriching the database in fermentation science, bio-energy studies and applications.

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