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# Ethanol production from cassava peels using *Saccharomyces cerevisiae* via ethanologenic fermentation process

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## Abstract

**Purpose** – This study aims to assess the effect of water variation on bioethanol production from cassava peels (CP) using *Saccharomyces cerevisiae* yeast as the ethanologenic agent.

**Design/methodology/approach** – The milled CP was divided into three treatment groups in a small-scale flask experiment where each 20 g CP was subjected to two-stage hydrolysis. Different amount of water was added to the fermentation process of CP. The fermented samples were collected every 24 h for various analyses.

**Findings** – The results of the fermentation revealed that the highest ethanol productivity and fermentation efficiency was obtained at 17.38 ± 0.30% and 0.139 ± 0.003 $gL^{-1}$ $h^{-1}$. The study affirmed that ethanol production was increased for the addition of water up to 35% for the CP hydrolysate process.

**Practical implications** – The finding of this study demonstrates that *S. cerevisiae* is the key player in industrial ethanol production among a variety of yeasts that produce ethanol through sugar fermentation. In order to design truly sustainable processes, it should be expanded to include a thorough analysis and the gradual scaling-up of this process to an industrial level.

**Originality/value** – This paper is an original research work dealing with bioethanol production from CP using *S. cerevisiae* microbe.

**Highlights**

(1) Hydrolysis of cassava peels using 13.1 M $H_2SO_4$ at 100 °C for 110 min gave high Glucose productivity
(2) Highest ethanol production was obtained at 72 h of fermentation using *Saccharomyces cerevisiae*
(3) Optimal bioethanol concentration and yield were obtained at a hydration level of 35% agitation
(4) Highest ethanol productivity and fermentation efficiency were 17.3%, 0.139 $g.L^{-1}.h^{-1}$

**Keywords** Bioenergy, Lignocellulosic biomass, Bioethanol, Glucose productivity, Fermentation

**Paper type** Research paper



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*Ethical issues:* The authors certify that this manuscript is the original work of the authors, and all data collected during the study are presented in this manuscript, and no data from the study has been or will be published separately elsewhere.

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## 1. Introduction

The exploration for sustainable energy is increased because of enhanced use of energy in different sectors including industrial, agricultural, domestic, transport and other commercial purposes (Al-Mamun *et al.*, 2023; Reza *et al.*, 2023; Onuoha, Nwafor, Igbokwe, & Aviara, 2019). However, the reliance on fossil fuels as the primary source of energy has contributed to the emergence of global issues such environmental degradation and global warming (Oseji, Ana, & Sokan-Adeaga, 2017; Sokan-Adeaga, Ana, & Sokan-Adeaga, 2015). These prompted the government, industry and energy sector to search for ecologically friendly, renewable and sustainable energy (Kiran, Kumar, & Deshmukh, 2014; Bolade, Ana, Lateef, & Sokan-Adeaga, 2019). Liquid biofuels were given top priority among renewable energy sources because they account for nearly 40% of global energy use (Demirbas, 2008). Biofuels help to reduce dependency on fossil fuels for sustainable development by addressing environmental concerns, fuel security and socioeconomic advantages (Shah *et al.*, 2023; Sathish & Singaravelu, 2020). Utilizing liquid biofuels helps with supply security, job creation, regional development and a decrease in greenhouse gas emissions (Bolade *et al.*, 2019; Sokan-Adeaga & Ana, 2015a).

The most common biofuel utilized in the transportation industry is bioethanol (Sokan-Adeaga *et al.*, 2015). Since the 1980s, there has been an increased interest in using bioethanol, and many nations have considered using it as an alternative fuel (Tan, Lee, & Mohamed, 2008). With a modest decline in production in 2012 and 2013, global ethanol production climbed from 13.12 billion gallons in 2007 to 25.68 billion gallons in 2015 (Renewable Fuels Association, 2015). The USA produced approximately 15 billion gallons of ethanol in 2015, making it the world's top producer. Eighty-five percent of the world's ethanol production comes from the USA and Brazil (Mohd Azhar *et al.*, 2017). In comparison to gasoline, bioethanol has a higher octane number, larger flammability limits, faster flames and greater heat of vaporization (Ana & Sokan-Adeaga, 2015). Bioethanol is less harmful, easily biodegradable and emits fewer airborne pollutants than petroleum fuel (Sokan-Adeaga, 2022). The manufacturing of bioethanol has utilized a range of feedstocks from the first, second and third generations. The first generation of bioethanol uses starchy feedstocks including corn, wheat, rice, potato, cassava, sweet potato and barley as well as sucrose-rich feedstocks like sugar cane, sugar beet, sweet sorghum and fruits. The consequent strains on land use and food security, in addition to the price associated with the feedstocks, have made this technique unpopular (Tshizanga, Aransiola, & Oyekola, 2017). Lignocellulosic biomass, such as wood, straw and grasses, is the source of second-generation bioethanol. Biomass-derived from lignocellulose is abundant, freely accessible and a potential resource for the creation of cost-effective alternative fuels (Sokan-Adeaga, Godson, & Olorunnisola, 2023). Microalgae and macroalgae biomass have been used to produce third-generation bioethanol (Nigam & Singh, 2011). With its potential to increase its biomass weight quickly, its ability to acquire lipids quickly, its year-round harvesting cycles and its ease of exploiting sunlight, water and $CO_2$, microalgae is a viable choice for biofuel production (Rafa, Ahmed, Badruddin, Mofijur, & Kamangar, 2021; Yin *et al.*, 2020). By converting a variety of carbohydrates to ethanol through fermentation, microorganisms like yeast play a crucial part in the creation of bioethanol. They are used in industrial plants because of their beneficial characteristics, including their high theoretical yield of ethanol (>90.0%), high tolerance and productivity levels of ethanol (>1.0 g/L/h), ability to grow on basic, inexpensive media and undiluted fermentation broth, resistance to inhibitors and ability to delay the effects of contaminants on growth conditions (Dien, Cotta, & Jeffries, 2003). Yeasts, the primary element in fermentation, have an impact on the output of ethanol.

There is currently a lot of penchant for bioethanol production as a sustainable bioenergy source by increasing the yield while lowering the cost of production through the use of low-

cost substrates, efficient fermentative organisms and process parameter modification (Sokan-Adeaga, Ana, Sokan-Adeaga, & Sokan-Adeaga, 2016). Biomass's availability and renewability are crucial for the manufacture of bioethanol. The largest source of renewable, possibly fermentable carbohydrates on earth is lignocellulosic biomass. Lignocelluloses are a substrate of significant biotechnological significance due to the chemical characteristics of their constituent parts (Sokan-Adeaga & Ana, 2018; Sokan-Adeaga, 2019).

Due to the amount of land and variety of biomass resources in Nigeria, bioenergy feedstock is not only plentiful but also widely accessible (Elijah, 2010). Nigeria produces the most cassava in the world and has the most oil palm farms, which is an excellent source of biodiesel (Abiodun, 2007). Due to their high dry matter content, high biomass production and ease of hydrolyzability, the non-food components of cassava have the potential to be extremely important in the energy generation process (Nuwamanya, Chiwona-Karltun, Kawuki, & Baguma, 2012). Given the massive amount of cassava peels (CP) produced nationwide, it is intriguing to note that Nigeria might also be a significant participant in the biofuel sector. Nevertheless, Nigeria has not been able to fully utilize these biomasses (Sokan-Adeaga & Ana, 2015b). Therefore, boosting the production and demand for bioethanol can be a substitute for conventional energy sources. This can be achieved through the development of technology that uses agricultural wastes as the only substrate for the production of bioethanol; however, this process has not been fully optimized (Adegbehingbe, Faparusi, & Adeleke, 2021).

Among the eukaryotes utilized in a wide range of industrial activities, including the manufacture of ethanol, *Saccharomyces cerevisiae* is without a doubt the most extensively researched (Parapouli, Vasileiadis, Afendra, & Hatziloukas, 2020). The yeast *S. cerevisiae* possesses a number of advantageous industrial traits, including quick development, effective anaerobic glucose metabolism, high ethanol production, excellent yield and strong tolerance to a variety of environmental stressors, including high ethanol concentrations, low pH and low oxygen levels (da Silva Fernandes *et al.*, 2022). Global alcohol production currently exceeds 100 billion liters per year, and the most common industrial microbe utilized to produce ethanol is *S. cerevisiae* (Walker & Walker, 2018). Even while the different *S. cerevisiae* strains employed in these procedures have adapted well, there is still a lot of room for improvement in terms of either maximizing the potential of already available strains or taking advantage of the vast natural reservoir of environmental isolates (Parapouli *et al.*, 2020). Therefore, using *Saccharomyces cerevisiae* as the ethanologenic organisms in our study, we investigated the impact of altering water content on the generation of bioethanol from CP. The novelty of this investigation is that it provides data on the effect of water on the ethanol production capacity of *S. cerevisiae* via Separate Hydrolysis and Co-Fermentation (SCHF) technique which hitherto is sparsely available thus enriching the existing database.

## 2. Materials and methods

### *2.1 Experimental design*

The substrate CP was collected from the cassava processing centre at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. A significant amount of the biomass was collected in polythene bag to carry out the experiment. The CP was air-dried until all moisture was removed. The dried CP was milled using a mortar and pestle and sieved with a +1.5 mm sieve to get homogeneous powder. The microbial fermentation of CP was carried out using *S. cerevisiae* as the ethanologenic microorganism to produce bioethanol. SHCF technique was also employed for bioethanol production. The experimental process consisted of biomass pre-treatment, chemical hydrolysis of biomass, neutralization process (to separate the sugars from acid), fermentation process and distillation of fermented products followed

by analysis (Sokan-Adeaga *et al.*, 2015; Bolade *et al.*, 2019; Ana & Sokan-Adeaga, 2015; Farone & Cuzens, 1996a, 1996b).

The microbial fermentation optimization phase was divided into three experimental groups as described by Hossain *et al.* (2011). This was done to assess the effect of varied degrees of hydration treatments on the total soluble solids (TSS), pH and bioethanol concentration of samples before and after fermentation at 30°C, respectively. An equal mass of 20 g of milled CP was utilised for each treatment group. The experimental plan is shown in Table 1.

### *2.2 Hydrolysis of the biomass*

A 100 ml of 13.1 M $H_2SO_4$ was mixed with 20 g of each of the milled CP separately (1:5 (w/v) ratio) in a glass container with a cover lid. The combination was heated at 100 °C for 60 min in a water bath to form a thick gel that was squeezed through a sieve to produce the first hydrolysate (acid-sugar stream). Thereafter, the second hydrolysis on the leftover solid at 100 °C was carried out for 50 min using the same quantity of acid as used in the first stage. This also produced a thick gel which was sieved to obtain the second hydrolysate. The two hydrolysates were combined and the total volume was recorded. The total hydrolysis time was 110 min. The reaction of lignocellulosic materials to reducing (acidic) sugar is shown in Eqn 1:

$$(C_6H_6O_5)_n \xrightarrow{+H_2O} (CH_2O)_n \quad (1)$$

*Lignocellulose* *Conc.* $H_2SO_4$ *Reducing sugar*

The reducing sugar obtained were neutralized with calcium hydroxide [$Ca(OH)_2$]aq solution to raise the pH to about 5.5 (Eqn. 2). With Whatman No. 1 filter paper, the solution was purified in order to obtain pure sugar solution. This was assessed qualitatively using the Fehling solution. Sample from the sugar solution was also taken for quantitative determination of total soluble solids (TSS), glucose and total reducing sugars (TRS) concentrations, respectively.

$$(C_6H_{12}O_6 - - H_2SO_4 \quad + \quad Ca(OH)_2 \longrightarrow C_6H_{12}O_6 + (CaSO_{4(PPT)}\ 2H_2O \quad (2)$$

*Hydrolysate* *Lime* *Free Sugar* *Gypsum* *Water*

### *2.3 Sugars Fermentation*

The fermentation process adopted in this study was the technique described by Hossain *et al.* [24]. The free sugar solution obtained (as described in Table 2) from the neutralization process was fermented with *S. cerevisiae* under aseptic conditions using the proportion of 3 g of yeast to 1 L of sugar solution (Eqn 3). Rehydration of the dry yeast was performed in a water bath at

| Experiment | Mode of treatment |
|---|---|
| Experiment (Bottle 1) 1 | 0% water content serve as control |
| Experiment (Bottle 2) 2 | 15% water content treatment |
| Experiment (Bottle 3) 3 | 25% water content treatment |
| Experiment (Bottle 4) 4 | 35% water content treatment |

**Source(s):** Authors work

**Table 1.** Experimental plan for the optimization of bioethanol production from Cassava peel

40°C and thereafter added to the sugar solution after cooling. The optimization process was carried out by adding various quantities of water content (0%, 15%, 25% and 35%) of equivalent volumes of sugar solutions to each treatment group (Bottle 1, 2, 3 and 4), respectively, with the aim to ascertain how hydration affects bioethanol production. Ethanol presence, ethanol concentration and yield were carried out by periodically taken samples from the fermenting broth every 24 h. After fermentation, the solutions' pH and TSS were also assessed.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{yeast} \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_2 \uparrow (g)} \quad (3)$$

Ethanol presence was confirmed by adding a few drops of acidified $KMnO_4$ solution to 1 ml of each of the fermented broths and heated to boil. The presence of ethanol is confirmed by the decolorization of $KMnO_4$ to a colorless liquid with the evolution of a pungent smell of ethanal.

The fermentation efficiency (FE) and ethanol productivity (EP) were calculated using the formula described by Zhu, Li, Gong, and Wang (2012) in Eqns 4-5:

$$\text{FE}\,(\%) = \frac{\textit{Ethanol concentration}\,(gL^{-1}) \times 1}{\textit{Cassava peels conc.} \times 51.52\% \times 1.1\,(gL^{-1}) \times 0.51} \times 100 \quad (4)$$

$$\text{Ethanol Productivity}\,(gL^{-1}h^{-1}) = \frac{\textit{Ethanol concentration}\,(gL^{-1})}{\textit{Fermentation time}\,(hr)} \quad (5)$$

Note: FE = Fermentation Efficiency.

Thus, the obtained bioethanol solution was distilled to achieve pure ethanol at 78°C using a distillation flask. The ethanol volume obtained from the distillation was measured and recorded.

### *2.4 Analytical assay*

The pH, TSS, glucose yield, TRS and bioethanol yield of the CP were analyzed. Physicochemical and proximate analyses of the produced ethanol were performed. pH meter was used to determine the pH of the solution. TSS was measured with the aid of a refractometer as described by Hossain *et al.* (2011). The Association of Official Analytical Chemists (AOAC) methods were used to calculate the concentrations of glucose and TRS (Association of Official Analytical Chemists (AOAC, 1984, 1990, 1998), 1984). The TRS content was quantitatively evaluated using the phenol-sulphuric acid method as highlighted by Dubois, Gilles, Hamilton, Rebers, and Smith (1956), while the glucose concentration was calculated using the Anthrone approach as defined by the A.O.A.C. (1984). The following equations (6) and (7) were used for the calculations of the original sample's glucose and TRS concentrations and yields.

**Table 2.** Mean volume of sugar solution obtained from the 13.1 M acid hydrolysis and subjected to fermentation

| Samples | Volume of sugar solution (ml) (Mean ± SD) |
|---|---|
| Bottle 1 | 161.00 ± 7.00 |
| Bottle 2 | 161.00 ± 1.00 |
| Bottle 3 | 163.66 ± 2.51 |
| Bottle 4 | 168.33 ± 1.52 |

**Source(s):** Authors work

$$Concentration\ (C_1) = \frac{Absorbance\ reading}{Gradient} \tag{6}$$

$$Yield = \frac{(C_1 \times 125) \times 1000}{D}\ mg \Big/ kg\ dry\ weight \tag{7}$$

where $C_2$= Concentration of reducing sugars (mg/ml) read from the appropriate calibration graph, 125 = Dilution factor, since 2 g of the analyte was dissolved in 250 ml of the distilled water, D = Dry weight of the original sample, which is 20 g., and 1000 = Conversion factor, to convert the final value from mg/g to mg/kg.

Ethanol concentration was quantitatively determined by UV Spectrophotometer using acidified potassium dichromate solution at a wavelength of 313 nm (AOAC, 2000, 2012; Sayyad, Chardhari, & Panda, 2015). The ethanol concentration and yield were calculated using Eqns 8 and 9.

% Ethanol (g/100ml) was calculated, viz.:

$$= \frac{(AbS - AbB) \times AG}{Volume\ of\ sample\ taken} \times 100 \tag{8}$$

$$\text{Ethanol Yield} = \frac{C \times V \times 50 \times 10^6}{Density\ of\ ethanol}\ \frac{ml}{kg}\ dry\ weight \tag{9}$$

where AbS = Absorbance of Sample, AbB = Absorbance of Blank, AG = Average Gradient, C = Concentration of ethanol (mg/ml) [multiply with $10^{-6}$ to convert from mg/ml to kg/ml, V = Volume of sugar (ml) and 50 = Multiplication factor used to extrapolate the result from the original sample of 20 to kg, Density of ethanol = 789 kg/$m^3$. $10^6$ = Conversion factor to convert final answer from $m^3$/kg to $cm^3$/kg (ml/kg).

## 3. Data analysis

The IBM Statistical Product and Service Solutions (IBM SPSS Statistics), version 27.0 software for descriptive and inferential statistics, was used to analyze the data. Descriptive statistics, including bar charts, line graphs, means and standard deviations, were used to summarize the data. The findings of the laboratory analyses were subjected to a one-way Analysis of Variance as stated by the Statistical Analysis System (1997) and a New Duncan's Multiple Range Test (1955) for means separation at the 95% level of probability.

## 4. Results

### *4.1 Hydrolysis results of the cassava peels*

The results for Glucose concentration, Glucose Hydrolysis Efficiency (GHE) %, Glucose Productivity, TRS concentration and TRS Productivity at the hydrolysis stage are shown in Figure 1. The overall mean Glucose and TRS concentrations obtained from the CP were 19.00 ± 0.19 and 35.92 ± 1.02 $gL^{-1}$ respectively. Likewise, the overall mean GHE %, Glucose Productivity and TRS productivity of the CP were 16.77 ± 0.07%, 10.37 ± 0.04 and 19.60 ± 0.56 $gL^{-1}h^{-1}$, respectively. All these results imply a promising yield of sugar production from CP using the described hydrolysis method.

### *4.2 Optimization studies*

Table 3 illustrates the effect of unequal amount of water treatments (0%, 15%, 25% and 35%) on the mean TSS, pH and bioethanol concentration of the CP hydrolysate. In Figure 2, the

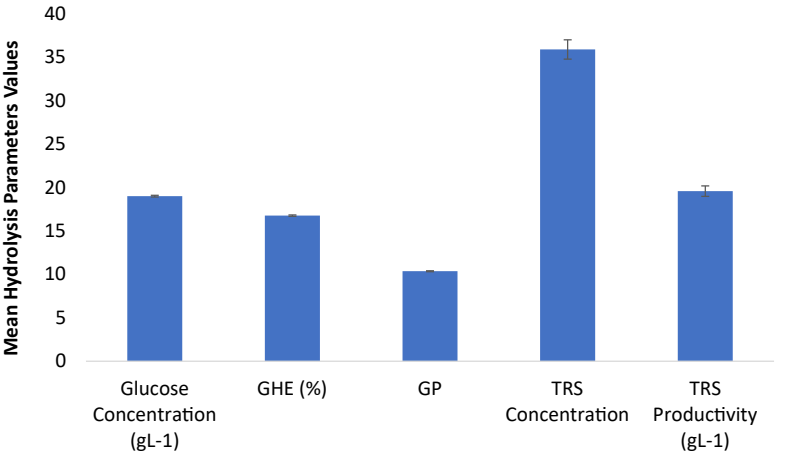



**Figure 1.**
Results of hydrolysis parameters

**Source(s):** Authors work

results from the fermented CP broths depicted that the mean bioethanol concentration varied between 0.89 and 1.00%. The lowest concentration of 0.89 ± 0.01% (w/v) was produced in Bottle 1 (control), i.e. without water, while the highest concentration of 1.00 ± 0.02% (w/v) was produced by Bottle 4 (35% of water). Bottle 2 (15% of water) and Bottle 3 (25% of water) had 0.91 ± 0.01% and 0.96 ± 0.01% of bioethanol concentrations, respectively. The bioethanol concentration varied directly to the water quantity added. The different quantities of water introduced to the CP hydrolysates gave different bioethanol concentration values, which were significant at $p < 0.05$.

As shown in Table 3, the mean TSS of the fermented cassava broths was lower than before fermentation ($p < 0.05$). Among the fermented CP broths, Bottle 1 exhibited the highest value of TSS, followed by Bottles 2, 3 and 4, respectively. Comparison of the TSS of the fermented CP broth across the different bottles was significant ($p < 0.05$). Also as shown in Table 3, the mean pH values prior to fermentation were greater compared to the post-fermentation readings ($p < 0.05$). At the end of fermentation, Bottle 4 had the highest pH compared to the others, followed by Bottles 3 and 2, with Bottle 1 having the lowest. These differences in the pH of the fermented CP broths across the different bottles were significant ($p < 0.05$).

*4.3 Effect of water treatment on glucose, TRS and bioethanol yields*

The effect of varied quantities of water (0%, 15%, 25% and 35%) on glucose, TRS and bioethanol yields from CP are depicted in Table 4 and Figure 3, respectively. It is evident in Table 4, that the mean glucose and TRS yield from the CP after fermentation were statistically insignificant ($p > 0.05$). The fermentation of Bottle 4 produced the highest bioethanol yield, followed by that of Bottle 3, Bottle 2 and Bottle 1, respectively ($p < 0.05$).

*4.4 Effect of water (%) content on fermentation efficiency and ethanol production*

The effects of water content variation (0%, 15%, 25% and 35%) on FE (%) and ethanol production ($gL^{-1}$ $h^{-1}$) of CP are shown in Figure 4. The highest mean FE and ethanol production was observed in Bottle 4 being 17.38 ± 0.30% and 0.139 ± 0.003 $gL^{-1}h^{-1}$, this was followed sequentially by Bottle 3 (16.56 ± 0.18%, 0.133 ± 0.002 $gL^{-1}h^{-1}$) and Bottle 2 (15.68 ± 0.17%, 0.126 ± 0.001 $gL^{-1}h^{-1}$), respectively, with Bottle 1 being the least (15.21 ± 0.06%, 0.123 ± 0.002 $gL^{-1}h^{-1}$). At p0.05, these outcomes were significantly different.

| Parameter (amount of water %) | Total soluble solids (TSS) g/100g (°Bx) | | pH | | Glucose concentration %(w/v) [g/100mL] | | | |
|---|---|---|---|---|---|---|---|---|
| | Initial | After | Initial | After | 0hr | 24hrs | 48hrs | 72hrs |
| Bottle 1 (0%) control | $27.35 \pm 0.12_b$ | $14.79 \pm 0.07_c$ | $5.50 \pm 0.00_a$ | $4.39 \pm 0.06_a$ | $1.90 \pm 0.01_a$ | $1.10 \pm 0.01_c$ | $0.32 \pm 0.01_a$ | $0.04 \pm 0.01_a$ |
| Bottle 2 (15%) | 27.36 ± 0.09b | 14.04 ± 0.19b | 5.50 ± 0.00a | 4.45 ± 0.05ab | 1.90 ± 0.01a | 1.09 ± 0.01bc | 0.31 ± 0.01a | 0.04 ± 0.00a |
| Bottle 3 (25%) | $27.20 \pm 0.08_{ab}$ | $8.84 \pm 0.09_a$ | $5.50 \pm 0.00_a$ | $4.54 \pm 0.05_b$ | $1.90 \pm 0.01_a$ | $1.07 \pm 0.01_{ab}$ | $0.31 \pm 0.01_a$ | $0.04 \pm 0.01_a$ |
| Bottle 4 (35%) | $27.04 \pm 0.10_a$ | $8.56 \pm 0.06_a$ | $5.50 \pm 0.00_a$ | $4.69 \pm 0.03_c$ | $1.90 \pm 0.01_a$ | $1.06 \pm 0.01_a$ | $0.31 \pm 0.01_a$ | $0.03 \pm 0.01_a$ |
| *F* value | 7.14 | 2504.87 | 0.00 | 21.83 | 0.00 | 10.19 | 2.22 | 2.22 |
| *p* value | *0.012 | *0.000 | 1.000 | *0.000 | 1.000 | *0.004 | 0.163 | 0.163 |

| Parameter (amount of water %) | Total reducing sugars concentration %(w/v) [g/100mL] | | | | Ethanol concentration %(w/v) [g/100mL] | | | |
|---|---|---|---|---|---|---|---|---|
| | 0hr | 24hrs | 48hrs | 72hrs | 0hr | 24hrs | 48hrs | 72hrs |
| Bottle 1 (0%) control | $3.59 \pm 0.11_a$ | $2.65 \pm 0.08_b$ | $1.54 \pm 0.09_b$ | $0.56 \pm 0.10_a$ | $0.00 \pm 0.00$ | $0.49 \pm 0.01_a$ | $0.52 \pm 0.01_a$ | $0.89 \pm 0.01_a$ |
| Bottle 2 (15%) | 3.56 ± 0.12a | 2.59 ± 0.09ab | 1.46 ± 0.06b | 0.53 ± 0.07a | 0.00 ± 0.00 | 0.50 ± 0.01a | 0.54 ± 0.01b | 0.91 ± 0.01a |
| Bottle 3 (25%) | $3.58 \pm 0.12_a$ | $2.51 \pm 0.05_{ab}$ | $1.38 \pm 0.06_{ab}$ | $0.49 \pm 0.08_a$ | $0.00 \pm 0.00$ | $0.52 \pm 0.01_b$ | $0.62 \pm 0.01_c$ | $0.96 \pm 0.01_b$ |
| Bottle 4 (35%) | $3.59 \pm 0.11_a$ | $2.44 \pm 0.07_a$ | $1.25 \pm 0.06_a$ | $0.40 \pm 0.09_a$ | $0.00 \pm 0.00$ | $0.54 \pm 0.01_c$ | $0.74 \pm 0.01_d$ | $1.00 \pm 0.02_c$ |
| *F* value | 0.05 | 4.84 | 9.38 | 1.98 | – | 59.83 | 589.50 | 57.57 |
| *p* value | 0.986 | *0.033 | *0.005 | 0.195 | – | *0.000 | *0.000 | *0.000 |

**Note(s):** Different letters (a, b, c and d) indicate significant differences along the columns
*Significant at $p = 0.05$
**Source(s):** Authors work

**Table 3.**
Effect of water quantity on the mean concentrations of bioethanol, total soluble solids and pH of Cassava peels (fermentation at 30°C) (Mean ± SD, n = 3)

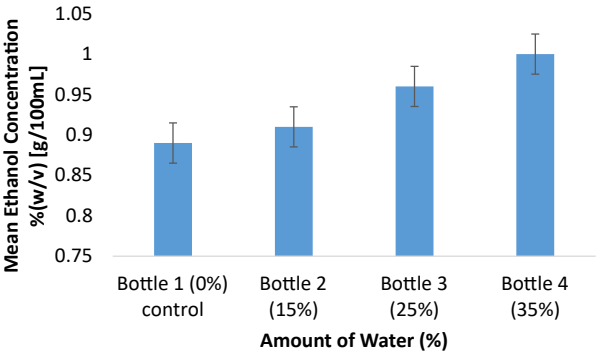



**Figure 2.**
Mean ethanol concentration obtained at various hydration levels

**Source(s):** Authors work

*4.5 Distillation results for laboratory studies*

The results of the mean volume of ethanol obtained from the different treatment groups (Bottle 1–4) are depicted in Figure 5(a). Bottle 4 gave the highest mean volume of ethanol recovery of 54.33 ± 4.01 ml while the lowest mean volume of 36.20 ± 1.50 ml was found in Bottle 1. The differences in the mean volume of ethanol recovered in the various treatment groups were statistically significant at $p < 0.05$. Likewise, the mean percentage ethanol concentration by volume (% v/v) obtained from the different treatment groups is depicted in Figure 5(b). The highest mean volume of ethanol concentration (% v/v) was obtained in Bottle 4 (30.67 ± 1.57 v/v) while the least value was obtained in Bottle 1 (28.49 ± 0.27 v/v) ($p < 0.05$). Last but not least, Figure 5(c) displayed the mean percentage ethanol yield achieved from the various treatment samples. Bottle 4 gave the best percentage ethanol yield of 54.78 ± 0.72% while the least value was found in Bottle 1 (48.25 ± 0.90%). At $p < 0.05$, these variations were determined to be significant.

## 5. Discussion

*Saccharomyces cerevisiae*, the key player in the industrial ethanol production among a variety of yeasts that produce ethanol through sugar fermentation, is essentially synonymous with the term "alcoholic fermentation" (Parapouli *et al.*, 2020). Under anaerobic conditions, *S. cerevisiae* uses glycolysis to catabolize sugars, reaching the step of pyruvic acid formation. In the process that follows, the latter is changed by pyruvate decarboxylase into acetaldehyde and carbon dioxide, which is then reduced to ethanol by alcohol dehydrogenase while simultaneously releasing $NAD^+$. As a result, the terminal step reactions that result in ethanol are crucial and serve as the foundation for significant fermentation industries (Walker, 2004).

CP is a cheap and affordable source of nutrients for the fermenting bacteria as well as energy for animals. Many carbohydrates (simple sugars) can be produced when a certain yeast strain ferments CP in a fermentation media (Nuwamanya *et al.*, 2012). Using *S. cerevisiae* to produce simple sugars from CP under controlled conditions will require process parameter optimization, which could increase bioethanol yield. This study led to the conclusion that CP might be used as a source of carbon energy for the yeast that is fermenting to produce ethanol (Chibuzor, Uyoh, & Igile, 2016). The selection of yeast for this study may have been influenced by its greater tolerance for acidic environments and low pH levels than other microbes, such as bacteria. It has been documented that *S. cerevisiae* can be isolated from fermented CP (Mariam, Manzoor, Ali, & Ul-Haq, 2009). Table 3 shows that the pH range of the CP hydrolysate before and after fermentation in this investigation dropped from 5.50 to 4.39,

| Parameter (amount of water %) | Glucose yield [g/kg] | | | Total reducing sugars (TRS) yield [g/kg] | | | Ethanol yield [mL/kg] | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0hr | 24hrs | 48hrs | 72hrs | 0hr | 24hrs | 48hrs | 72hrs | 0hr | 24hrs | 48hrs | 72hrs |
| Bottle 1 (0%) Control | $118.70 \pm 0.49_a$ | $68.58 \pm 0.41_c$ | $19.88 \pm 0.29_a$ | $2.77 \pm 0.38_a$ | $224.27 \pm 7.83_a$ | $165.35 \pm 4.99_b$ | $96.44 \pm 5.99_b$ | $35.13 \pm 6.34_a$ | $0.00 \pm 0.00$ | $48.18 \pm 1.17_a$ | $50.98 \pm 1.41_a$ | $87.31 \pm 2.44_a$ |
| Bottle 2 (15%) | $118.64 \pm 0.36_a$ | $67.85 \pm 0.52_{bc}$ | $19.56 \pm 0.33_a$ | $2.46 \pm 0.28_a$ | $222.44 \pm 7.81_a$ | $161.98 \pm 5.36_{ab}$ | $91.48 \pm 4.00_b$ | $32.71 \pm 4.52_a$ | $0.00 \pm 0.00$ | $50.78 \pm 0.88_a$ | $55.03 \pm 1.11_b$ | $92.44 \pm 1.55_a$ |
| Bottle 3 (25%) | $118.73 \pm 0.46_a$ | $67.04 \pm 0.54_{ab}$ | $19.36 \pm 0.25_a$ | $2.23 \pm 0.31_a$ | $223.92 \pm 7.69_a$ | $157.00 \pm 2.91_{ab}$ | $85.98 \pm 3.52_{ab}$ | $30.67 \pm 5.13_a$ | $0.00 \pm 0.00$ | $54.11 \pm 1.45_b$ | $63.87 \pm 1.70_c$ | $99.30 \pm 2.62_c$ |
| Bottle 4 (35%) | $118.69 \pm 0.39_a$ | $66.31 \pm 0.60_a$ | $19.15 \pm 0.28_a$ | $1.96 \pm 0.38_a$ | $224.46 \pm 6.87_a$ | $152.40 \pm 4.09_a$ | $78.33 \pm 4.03_a$ | $24.67 \pm 5.76_a$ | $0.00 \pm 0.00$ | $57.96 \pm 1.22_c$ | $78.59 \pm 1.46_d$ | $107.22 \pm 2.86_d$ |
| $F$ value | 0.20 | 10.68 | 3.44 | 3.04 | 0.64 | 4.91 | 8.99 | 2.00 | – | 37.68 | 217.79 | 38.24 |
| $p$ value | 0.996 | *0.004 | 0.072 | 0.093 | 0.612 | *0.032 | *0.006 | 0.193 | – | *0.000 | *0.000 | *0.000 |

**Note(s):** Different letters (a, b, c and d) indicate significant differences along the columns
*Significant at $p = 0.05$
**Source(s):** Authors work

**Table 4.**
Effect of water treatments on the glucose, total reducing sugars and bioethanol yields of Cassava peels (fermentation at 30°C) (Mean ± SD, n = 3)

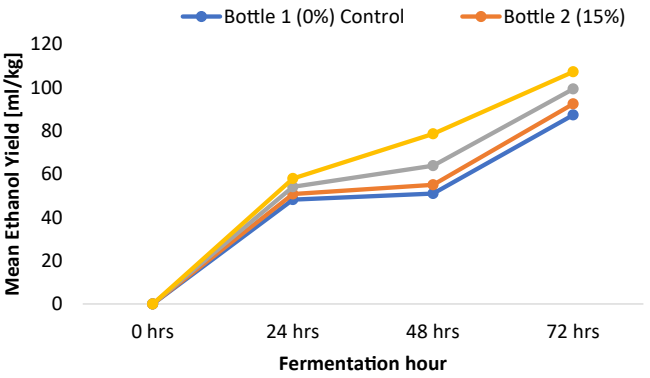



**Figure 3.**
Comparison of mean bioethanol yield of cassava peels for different water quantity

**Source(s):** Authors work

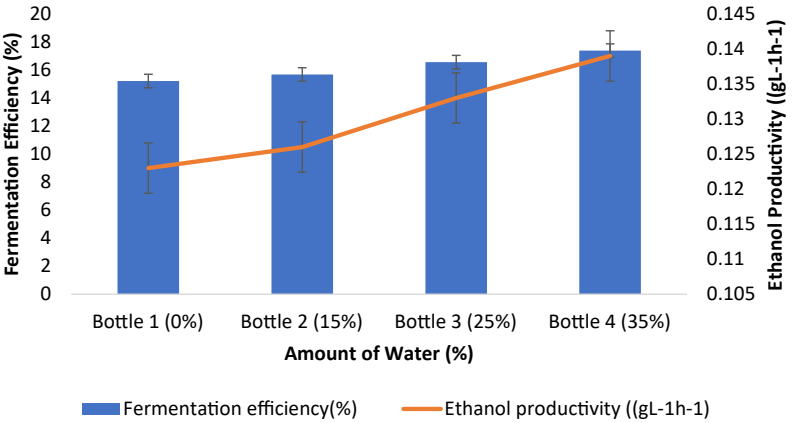
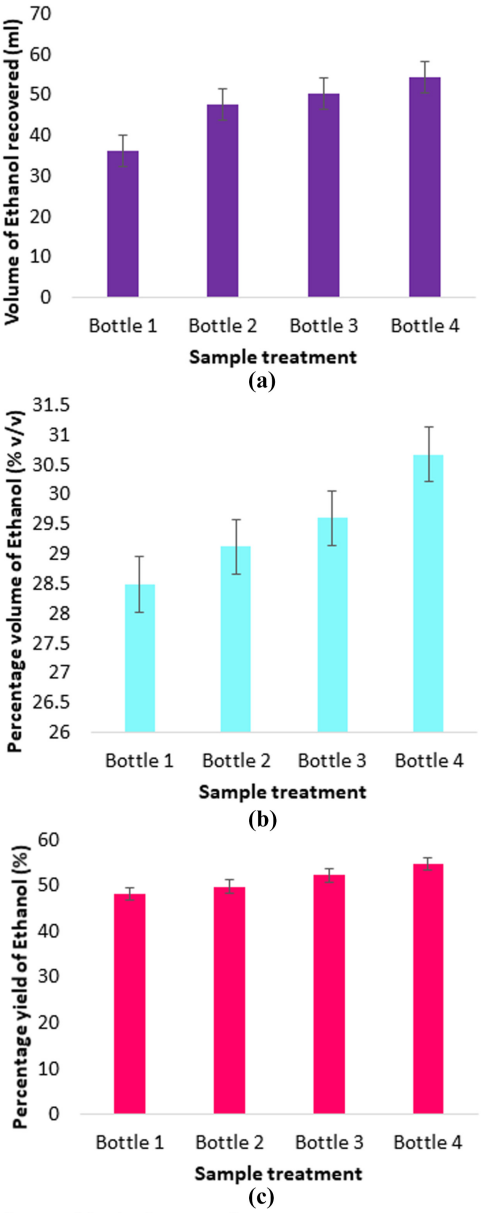



**Figure 4.**
Effect of water content variation on fermentation efficiency and ethanol production of cassava peels in the laboratory studies

**Source(s):** Authors work

which remains within the acidic medium. The subsequent mixed fermentation process that the microorganisms went through and their capacity to make organic acids (acetic acid, lactic acid and succinic acid) in the fermentation medium may be what caused the pH value to decrease. Additionally, the pH can be lowered by the anaerobic conditions that the fermenting yeast creates in the fermenters (Adeleke, Akinyele, Olaniyi, & Jeff-Agboola, 2017; Adegbehingbe *et al.*, 2021). Due to its acidophilic nature, yeast often grows better in acidic environments. A pH range of 4.0 to 6.0 is ideal for its growth. Since the activity of biological components including enzymes, transport proteins and proteins bound to plasma membranes depends on optimum pH which is influenced by the presence of oxygen, temperature and yeast strain (Narendranath & Power, 2005).

Also, as evident in Tables 3 and 4, there is a rapid reduction in the glucose and TRS concentrations and yields during the fermentation period of all the treatment groups (Bottle 1–4). The decrease in reducing sugar in the yeast-inoculated CP can be attributed to the microorganisms' (*S. cerevisiae*) usage of the sugar as a carbon source for development and subsequent ethanol production (Adegbehingbe *et al.*, 2021). The results of this experiment were consistent with those of Adegunloye and Udenze (2017), who demonstrated a decrease




**Source(s):** Authors work

**Figure 5.**
(a) Mean volume of ethanol recovered (ml), (b) Mean percentage volume of ethanol and (c) Mean percentage yield of ethanol from the various sample treatments

in the TRS of co-culture fermented cocoyam peel. The authors also discussed how *S. cerevisiae*'s fast conversion of sugar to ethanol during the fermentation process might gradually reduce the sugar concentration to prevent feedback suppression of some molds' amylolytic activity at the start of the fermentation. Additionally, the inoculation of cell-free

CP hydrolysate with *S. cerevisiae* after being cultured for 72 h resulted in the highest amount of ethanol production in all the treatment groups (Bottle 1–4) as depicted in Figure 3 and Tables 3 and 4, respectively. The outcomes of this investigation are also in agreement with Suryawati *et al.* (2008) and Faga, Wilkins, and Banat (2010), who discovered that 72 h was the optimum amount of time for several strains of thermotolerant yeasts to produce the maximum ethanol. According to a significant amount of research that is available, optimal bioethanol synthesis from a variety of feedstocks is accomplished after 72 h of fermentation (Kim *et al.*, 2014; Karagoz & Ozkan, 2014).

Essentially, water is necessary for the fermentation process. Hypertonic sugar media significantly affect cell physiology, which can lead to osmotic stress (reduced water availability) on cells. Water activity (aw) in *S. cerevisiae* cells must be high and must be at least 0.65. Yeast cells can overproduce glycerol or other osmolytes, such as trehalose, in response to a lack of moisture, acting as a buffer to prevent yeast cells from dehydrating (Graeme & Graham, 2016). These substances can efficiently replenish cell volume, replace lost cellular water and support the continuation of yeast metabolism. Nevertheless, excessive glycerol production could result in lower ethanol yields in elevated gravity fermentations. This study evaluated the impact of water content on the ethanol generation of CP at four different levels: 0% (control), 15%, 25% and 35%. According to the findings, which are represented in Figures 2, 3 and 5; Tables 3 and 4, the samples handled with the maximum water volume (35%) in the laboratory investigation produced the highest percentage volumes, concentrations and yields of ethanol. Thus, suffice it to say that in the laboratory investigation, a water content of 35% was ideal for the efficient generation of ethanol from CP at a fermentation temperature of 30 °C. It is obvious that a rise in water volume causes a rise in bioethanol concentration. This conclusion is supported by Hossain *et al.* (2011)'s research, which produced a comparable outcome using fermented, rotten banana marsh. The sample that was treated with 35% water during fermentation at 35°C produced the highest amount of ethanol, according to the authors.

The results of the fermentation stage revealed the FE and EP obtained from the 20% CP at 72 h of fermentation for the various water treatments. The results obtained for these parameters from the laboratory study (as illustrated in Figure 4) show that Bottle 4 (35% water treatment) produced the highest FE and EP, respectively. This implies that the 35% hydration treatment provided an enabling environment that enhanced the fermenting efficiency and ethanol productivity of the yeast (*S. cerevisiae*). The highest ethanol concentration $1.00 \pm 0.02$ g/100 ml ($10.00 \pm 0.2$ $gL^{-1}$) and FE ($17.38 \pm 0.30$ %) obtained in this current work were however less than the values reported by Zhu *et al.* (2012) and Kosugi *et al.* (2009), respectively, using the separate hydrolysis and fermentation process. Also, deducing from Figures 5a, 5b and 5c, the peak mean volume of ethanol recovery was $54.33 \pm 4.01$ ml, while the highest percentage ethanol concentration by volume was $30.67 \pm 1.57$ % (v/v) corresponding to a maximum percentage yield of $54.78 \pm 0.72$ % of the theoretical conversion after distillation. These figures were higher than the ethanol concentrations reported for various tuber crops, namely cassava, sweet potato, potato, yam, aroids, sugar beet, etc. *by the yeast, S. cerevisiae*, according to Thatoi, Dash, Mohapatra, and Swain (2014). The results of this experiment were also more significant than those of Adegunloye and Udenze (2017), who found that cocoyam peel fermented with *A. niger* and *S. cerevisiae* produced an ethanol yield of 6% at its highest. However, the value obtained in the present work was lower compared to the peak ethanol concentration of 41 % (v/v) and peak yield of $14.46 \pm 2.08$ $g/cm^3$ given by Chibuzor *et al.* (2016) when CP cultivar TME 4779 was treated with *Rhizopus nigricans, Spirogyra africana* and *S. cerevisiae*, respectively.

According to Oyeleke, Dauda, Oyewole, Okoliegbe, and Ojebode (2012), the highest bioethanol yield can be obtained from CP by hydrolyzing it with *Pleurotus ostreatus* and *Gloeophyllum sepiarium* and fermenting it with *S. cerevisiae* and *Zymomonas mobilis* to produce bioethanol. Akponah and Akpomie (2011) also showed that hydrolysis of CP with acid prior to fermentation by *S. cerevisiae* gave the highest yield of bioethanol of 17.52% v/w ethanol,

compared to ethanol yield produced from enzyme hydrolysis (4.07% v/w) at 24 h of fermentation time and amylolytic fungi (10.5% v/w). A report by Abidin, Saraswati, and Naid (2014), on the optimal fermentation timing of CP, depicted that the optimal fermentation time of CP hydrolyzed by 0.5 M sulphuric acid solution was 4 days which resulted in 3.58% v/v bioethanol produced. Abdelrahman, Christopher, Gregory, and Chenyu (2020) improved the productivity of bioethanol production using marine yeast and seawater-based media. The authors reported that *S. cerevisiae* AZ65 grew well in media containing up to 10.5% (w/v) sea salts and 20% (w/v) glucose compared with an industrial distiller's strain, *S. cerevisiae* NCYC2592. A multi-stage batch fermentation process was also investigated to increase ethanol productivity. Two different seawater-based media were used: SW-YPD medium and SW-molasses medium. *S. cerevisiae* AZ65 achieved an ethanol concentration of 113.52 $gL^{-1}$ with a productivity of 4.15 g $L^{-1}h^{-1}$ using SW-YPD medium and an ethanol concentration of 50.32 $gL^{-1}$ with a productivity of 2.46 g $L^{-1}h^{-1}$ using SW-molasses medium. These results confirmed the potential of seawater and marine yeasts for implementation in the bioethanol industry using a multi-stage fermentation process. Other authors have demonstrated the of marine yeast strains for bioethanol production using freshwater (O'Neil *et al.*, 2014; Spang, Moomaw, Gallagher, Kirshen, & Marks, 2014).

Despite the fact that *S. cerevisiae* is the most common sugar fermenter, other yeast species can also produce bioethanol from sugar fermentation (Walker & Walker, 2018). Among other uses, the generation of bioethanol from polyfructan substrates has been studied by *Kluyveromyces marxianus* (Flores *et al.*, 2013). The use of yeasts that are tolerant to inhibitors would improve the efficiency of ethanol production on an industrial scale (Tofighi, Assadi, Asadirad, & Karizi, 2014), and the frequent difficulties of yeasts can be overcome by utilizing yeast that is tolerant to ethanol and thermostability. Using hybrid, genetically modified, or co-cultures of two yeast strains, pentose fermentation issues can be resolved. Pentose and hexose carbohydrates are concurrently fermented to ethanol using hybrid yeast strains. The hybrid strain was created by combining *S. cerevisiae* protoplast with xylose-fermenting yeast protoplast from *P. tannophilus*, *C. shehatae* and *P. stipitis* (Kumari & Pramanik, 2013). High-yield bioethanol production from xylose has been achieved using genetically modified *S. cerevisiae* and co-culture of two strains. The recombinant DNA technique is used in genetic engineering to up-regulate stress tolerance genes in order to overcome restrictive circumstances (Dogan, Demirci, Aytekin, & Sahin, 2014).

### *5.1 Limitations of the study*

Although *S. cerevisiae* was able to optimize changes in hydration level utilizing the SHCF approach to produce a highly promising yield of bioethanol from CP, this study still has a number of drawbacks. First off, the study exclusively uses CP as the carbon substrate, which could restrict how broadly the results can be applied to other substrates. Second, the study fails to examine the impact of other production techniques, Simultaneously Saccharification and Co-Fermentation (SSCF) process; and production factors, such as temperature, sugar concentration, pH, fermentation time, agitation rate and inoculum size, on yeast fermentation effectiveness and ethanol production. However, the work adds to the body of knowledge on the generation of biofuels by examining the impact of hydration level on the effectiveness of fermentation and ethanol production by *S. cerevisiae* in a mixture of hexose and pentose sugars. Additionally, the study offers particular conclusions on the ideal level of hydration for optimizing the yield of ethanol from CP, which may be helpful for industrial uses.

## 6. Conclusion

The effective application in the present study of *S. cerevisiae* as an ethanologenic agent to ferment efficiently CP hydrolysate leading to ethanol production was further corroborated.

For small-scale flask studies, the influence of the fermentation parameter (change in water content) was examined, and the ideal condition was established. The study affirmed that the water treatment of the CP hydrolysate gave the increasing ethanol concentration, ethanol yield and fermentation efficiency among the various treatment groups and the highest amount was achieved from 35% of water. For the same concentration of water, the maximum efficiency of fermentation ($17.38 \pm 0.30\%$) and the highest amount of ethanol production ($0.139 \pm 0.003\ gL^{-1}h^{-1}$) was achieved. As a result, this finding demonstrates that in order to design truly sustainable processes, research should go beyond simply identifying promising biocatalysts and figuring out the ideal conditions. Instead, it should be expanded to include a thorough analysis and the gradual scaling-up of this process to an industrial level.

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