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# Bioethanol Production from Cassava Peels Inoculated with *Saccharomyces cerevisiae* and *Zymomonas mobilis*

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author KTA designed the study, performed the microbiological analysis, wrote the protocol and first draft of the manuscript. Authors FF and BSA managed the literature searches and analyses of the study. Authors KTA and BSA reviewed the drafts and proofread the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** This study was designed to determine the bioethanol production from fermented cassava peel using *Saccharomyces cerevisiae* and *Zymomonas mobilis*.
**Methodology:** Cassava peels were collected from cassava processing sites, washed, surface sterilized, dried, milled into flour, pretreated, and fermented. *Saccharomyces cerevisiae* and *Z. mobilis* suspensions were aseptically inoculated into the fermenting medium and allowed to stand for seven days. The pH, total reducing sugar, chemical composition, and bioethanol composition of the fermenting substrates were determined.
**Results:** A pH decrease from 5.2 to 4.1 was recorded in the sample fermented with *S. cerevisiae* while the least pH value of 3.8 was obtained from the sample fermented with *Z. mobilis,*

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respectively. The total reducing sugar (glucose) of fermented samples decreased from 3.4% to 1.5% (*Z. mobilis*) and 3.4% to 1.88% (*S. cerevisiae*) compared with the control sample. The chemical composition showed high protein and fat contents in the fermented samples. High percentage yield of 30% with ethanol volume of 45 mL was recovered from cassava peel inoculated with *S. cerevisiae* while flash point, i.e. the lowest temperature at which fuel produces enough vapor to cause ignition leading to flame generation of 24$^{o}$C, was recorded for both fermented samples inoculated with *S. cerevisiae* and *Z. mobilis*.

**Conclusion:** The ability of the bacterium and the yeast isolates exhibiting high potential for bioethanol production could be promising in various industrial processes as an alternative to fossil transportation fuel.

*Keywords: Agricultural waste; anti-nutrient; bioethanol; chemical analysis; fermenting substrate.*

## 1. INTRODUCTION

The poor power supply and energy crisis are the major problems facing the world that demand urgent attention. Our society is faced with a lot of energy crises; such that, as the human population increases, the rate of energy consumption also increases. Proper management and sustainable future energy production, therefore, become imperative [1]. Currently, fossil fuels account for over 85% of the world's energy supply and their continuous use has negatively caused adverse effects on the environment due to partial internal combustion in the locomotive engine releasing carbon dioxide that results in global warming [2]. The limitation surrounding the fossil fuel supply and its environmental implications has prompted research focus in devising a long-term solution to energy challenges and power supply to meet industrial and domestic needs [3]. The quest by many countries for energy independence as well as widespread awareness on the need to reduce greenhouse gas emissions, has heightened the search for alternative energy sources from organic materials [4].

Biofuels are expected to reduce over dependence on imported petroleum associated with political and economic vulnerability, greenhouse gas emission, and other pollutants, and revitalization of world economy by increasing demand and prices of agricultural products [5]. Thus, increasing the demand for bioethanol can serve as an alternative source of energy, although, Nigeria currently depends on the importation of ethanol to meet its local demand. The swift changes in industrialization using agricultural wastes as a sole substrate in renewable energy generation has reduced over-dependence on fossil fuel, nevertheless, this technology has not been properly harnessed.

Over the years, fermentable agricultural wastes, such as orange peels, cassava peels, cow dung, cassava wastewater, etc. have been employed in producing biogas and bioethanol with success recorded [6]. Generally, indiscriminate discharge of wastes generated from homes, industries, and farm settlements constituting a nuisance affects environment and its ecological components. Some of these wastes consist lignocellulosic materials, which can easily degraded by enzymatic processes.

Under controlled fermentation, the biochemical process facilitated by the yeast activities in the biotransformation of lignin-containing plant materials to produce sugar and ethanol has yielded desirable end products [7]. Bioethanol production from plant materials containing glucose (i.e. sugarcane, corn, sugar beet), cereals (i.e. maize), and barley are known [8]. The advancement in biotechnology has revealed bioethanol production from livestock feeds, such as bagasse, miscanthus, brew spent grains, sorghum, switchgrass, reed canary grass, cord grasses, hemp, kenaf, potatoes, sweet potatoes, cassava, sunflower, fruits, molasses, stover, wheat, and Jerusalem artichoke [9]. Also, the use of corncob, cornstalk, cornhusk, sugarcane molasses, spent grains, and other agro-wastes can as well be substituted for edible food materials in the production of bioethanol [10].

In this current research, we focused on bioethanol production from complex substrate cassava (*Manihot esculenta*) peels. Cassava peels are an agricultural waste usually generated from cassava processing sites or cassava farmland. Other cassava products, such as *garri* or *elubo, fufu,* and *lafun* obtained during cassava processing have been highlighted [11]. Cassava peels contain high cyanogenic glycosides and if not properly detoxified by soaking, drying, and scraping before being consumed it can result in

cyanide poisoning. Similarly, cyanide content in cassava can affect ethanol production. A study by Jos and Kumoro [12] demonstrated the comparative effect of simultaneous saccharification and fermentation of sweet and bitter cassava using *S. cerevisiae* for bioethanol production. Furthermore, the authors accounted high ethanol production in sweet cassava compared to bitter cassava. Based on the classification of cassava cultivars, bitter cassava usually contain high cyanide while as low cyanide content in sweet cassava [13]. Usually, cassava peels contain starch, a relatively abundant renewable energy source, cheap, and easy to access for the production of fermentable glucose syrups and dextrins [11].

Cassava is cultivated and produced in large quantities in the tropical and subtropical regions, mainly in Asia, South America, and Africa. Processed cassava products can serve as excellent sources of carbohydrate foods for humans, while cassava peels constitute major components of animal feeds; both are good raw materials in industry in the production of starch with excellent characteristics; whitish, odorless, and tasteless and when cooked, yielding high paste viscosity, clarity, and stability [14]. They can also be used as a stabilizer in the food, paper, textile, pharmaceutical, and medicine industries [15].

In some developing countries, efforts are being directed towards transforming organic wastes into wealth by employing biotechnology approaches in the biotransformation of environmental wastes to reduce pollution problems [11]. Biotransformation of organic pollutants can be facilitated by microbial activities in the conversion of these wastes into useful products. This technology still suffers a setback in Nigeria due to lack of essential infrastructural tools and farmer's awareness on the potential and economic relevance of cassava waste in bioethanol production [9]. Bioethanol has been produced using batch fermentation process in an anaerobic generation plant inoculated with fungi strains, such as *Aspergillus niger*, *Mucor mucedo,* and *Saccharomyces cerevisiae* [16].

The basic procedural steps involved in bioethanol production include: (i) hydrolysis i.e. the conversion of cellulosic materials of the biomass into fermentable sugars, (ii) fermentation i.e. activities of microorganisms in the conversion of sugar into alcohol, and (iii) distillation i.e. recovery of bioethanol from the fermentable substrates. Bioethanol production in a fermentation medium can be charged with free or immobilized cells [17]. Cell mobilization in the fermentation medium can be employed in enhancing bioethanol yield due to ease separation of cell mass from the bulk liquid, reduced risk of contamination, optimization of operational stability, and cell viability throughout the operation system. Immobilized cells are easily entrapped within the polymeric matrices, such as agar-agar, calcium alginate, gelatin, and k-carrageenan. The most suitable carriers for cell immobilization are entrapment in calcium alginate beads due to simple technology, cost-effectiveness, and non-toxic [18]. Improving the bioethanol yields from fermentable substrates can be achieved by single or combined microbial isolates under controlled fermentation by optimization of process parameters and adjustment where necessary by supplying adequate nutrients to the fermenting microorganisms. Therefore, fermentation of pretreated cassava peels with *S. cerevisiae* and *Z. mobilis* for bioethanol production was performed.

## 2. METHODOLOGY

### 2.1 Source and Collection of Sample

Fresh cassava peels were collected from a local cassava processing factory in Akungba-Akoko, Ondo State, Nigeria in sterile zip-lock bags and then transported to the Microbiology laboratory for further microbiological and chemical analyses. The *Z. mobilis* isolated from fermented cassava flour, and *S. cerevisiae* (baker's yeast) obtained from Ibaka market, Akungba-Akoko, Ondo State, Nigeria were used for this study. In brief, isolation of *Z. mobilis* and *S. cerevisiae* from the fermenting cassava water and palm wine were performed by serially diluted 1 mL of the sample into 9 mL sterile distilled inside test tubes up to the appropriate dilutions ($10^6$). From the diluents, 0.1 mL was aseptically pipetted and dispensed into sterile Petri dishes and then pour plated with sterilized molten nutrient agar and yeast extract agar, respectively. The Petri plates were incubated at 37±2$^o$C for 18 hours for bacteria and 28±2$^o$C for 48 hours for yeast. The emerged colonies from the agar plates were sub-cultured to obtain a pure culture. Pure bacteria and yeast isolates were presumptively identified using morphological examinations, sugar fermentation, and some biochemical tests according to taxonomic indices described in

Bergey's Manual of Determinative Bacteriology [19].

## 2.2 Preparation and Pretreatment of Cassava Peels

Cassava peels were washed thoroughly under running tap water to remove the dirt; surface sterilized with 3% hypochlorite and later washed with sterile distilled water. The samples were air-dried and milled into powder before pretreatment by pasteurization in a hot water bath at 72$^{o}$C for 30 minutes and then ready for fermentation.

## 2.3 Fermentation Process

Ten milliliters (10 mL) of microbial suspensions each (*S. cerevisiae* and *Z. mobilis*) were inoculated into separate fermenters containing 500 mL pre-treated cassava peels mixture while un-inoculated fermenters served as control. The content of each fermenter was allowed to stand for 7 days, while samples were withdrawn at intervals of 24, 72, 120, 168, and 216 hours for further analyses.

## 2.4 Reducing Sugar and pH Determination

The total reducing sugar was determined in line with the standard methods of AOAC [20]. Approximately 50 grams of the pretreated cassava peels were weighed into 500mL calibrated conical flasks containing sterile distilled water. The medium was inoculated with suspension of *S. cerevisiae* and *Z. mobilis* and then allowed to stand for seven days at room temperatures. The pH of the medium was measured daily during the fermentation process.

## 2.5 Determination of Ethanol Production

The distillation method was used for the determination of ethanol production. The distillate obtained was re-distilled and percent ethanol was estimated using the method of Dias*, et al.* [21].

$$\% \text{ Ethanol (v/v)} = \frac{\text{Volume of distillate} \times 100}{\text{Volume of the fermentation mixture}}$$

## 2.6 Determination of the Chemical Composition of Cassava Peels

The chemical composition of cassava peels was determined in line with the standard methods of AOAC [20],and the parameters analyzed were protein, lipid, starch, reducing sugar, and cyanide.

## 2.7 Statistical Analysis

All analyses were carried out in triplicates on SPSS and Excel. Analysis of variance (ANOVA) plus Duncan's Multiple Range Test (DMRT) was used for comparison of means. Significance was accepted at $p < 0.05$.

# 3. RESULTS

## 3.1 pH of the Fermenting Cassava Peel Samples

Changes in pH of the fermenting mixture inoculated with *S. cerevisiae* and *Z. mobilis* during the fermentation process and control sample were shown in Fig. 1. A pH decrease was observed in all the samples throughout the fermentation process. There were no significant changes in the pH value of 5.2 in all the samples at day 0. As the fermentation progressed, pH of the fermenting mixture inoculated with *S. cerevisiae* and *Z. mobilis* tended to be lower compared to the control sample. A pH decreased from 5.2 to 4.1 in the sample fermented with *S. cerevisiae* with the least pH value of 3.8 were recorded from the sample fermented with *Z. mobilis* respectively.

## 3.2 Changes in Reducing Sugar of the Fermenting Cassava Peel Samples

Fig. 2 showed the total reducing sugars of the fermented substrate. The total reducing sugar (TRS) remained unchanged at day 0. The sample fermented with *Z. mobilis* showed a decrease in TRS from 3.4% to 1.6%, while sample fermented with *S. cerevisiae* showed a decrease from 3.4% to 1.5% compared with the control sample.

## 3.3 Chemical Composition of Fermenting Cassava Peel Samples

The chemical composition and reducing sugar contents of cassava peels were presented in Table 1. The reduction in starch, reducing sugar and cyanide contents of the sample fermented with *S. cerevisiae and Z. mobilis* were observed compared with the control. High protein and lipid contents of 3.3% and 2.1% were recorded from the samples fermented with *S. cerevisiae* compared with the control. The cyanide contents

of the fermented sample reduced considerably. There were significant differences in the carbohydrate contents of the fermented sample compared to the control sample.

### 3.4 Bioethanol Produced from Cassava Peel Samples

Table 2 showed the bioethanol produced from cassava peels. A high yield of ethanol (30%) with ethanol volume 45 mL was recovered from the fermented sample inoculated with *S. cerevisiae.* There were variations in the final volume of ethanol produced, while flash point of 24$^{o}$C was recorded for both samples inoculated with *S. cerevisiae* and *Z. mobilis*.

## 4. DISCUSSION

The previously characterized *S. cerevisiae* and *Z. mobilis* isolated from cassava flour and palm wine were used to ferment the cassava peels for bioethanol production; this evidently brought about changes in the pH and chemical composition of the fermented cassava peels. Cassava is classified into tuberous crops and propagated by stem. At maturity, harvesting and processing of cassava generate enormous waste used livestock feeding, and immoderate quality control and escape to the environment result in soil and water pollution [22].

Cassava peel is easily accessible, readily available as a cheap source of energy for livestock and nutrients for the fermenting microbes. Fermentation of cassava peel by a certain group of yeast in a fermentation medium can yield considerable amounts of carbohydrates (simple sugars) [23]. The need to optimize process parameters under controlled conditions using *S. cerevisiae* for the production of simple sugars from cassava peel may improve bioethanol yield. Hence, from this study, it was deduced that cassava peel may serve as a source of carbon energy for the fermenting yeast to produce ethanol [24]. The presence of some group of microorganisms in a fermentation medium might be due to their ability to utilize nutrients available in the fermenting substrate and also ability to secrete certain metabolites that inhibit the growth of competing pathogenic microbes. The choice of yeast in this study might be due to their tolerance to acidic conditions and low pH more than other microorganisms, such as bacteria. Isolation of *S. cerevisiae* from the fermented cassava peel has been reported [25].

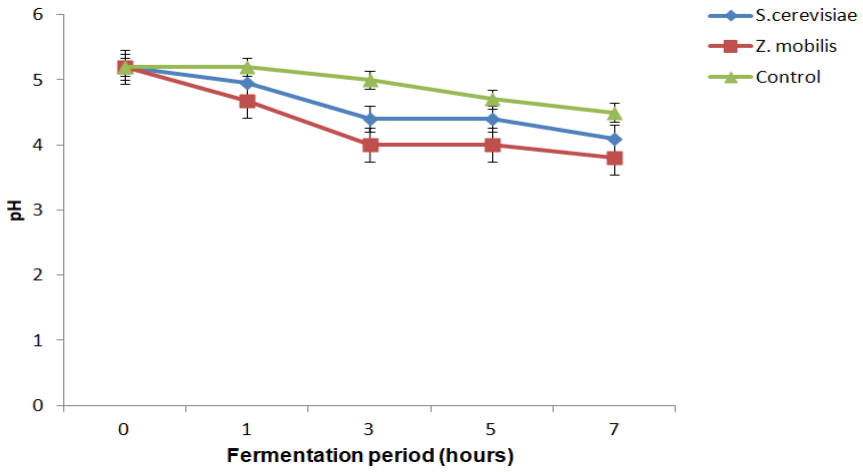


**Fig. 1. Changes in pH of the fermenting cassava peel samples**

**Table 1. Chemical composition and reducing sugar contents of fermented cassava peels**

| Component | *S. cerevisiae* | *Z. mobilis* | Control |
|---|---|---|---|
| Protein | 3.3$^{d}$ | 2.6$^{c}$ | 1.2$^{d}$ |
| Lipid | 2.1$^{c}$ | 1.8$^{d}$ | 0.9$^{e}$ |
| Carbohydrate | 56.1$^{a}$ | 59.2$^{a}$ | 70.5$^{a}$ |
| Reducing sugar (glucose) | 2.2$^{c}$ | 2.4$^{c}$ | 3.4$^{c}$ |
| Cyanide | 10.9$^{b}$ | 11.1$^{b}$ | 24.0$^{b}$ |

*Mean values with different superscripts within the same column represent a significant difference*

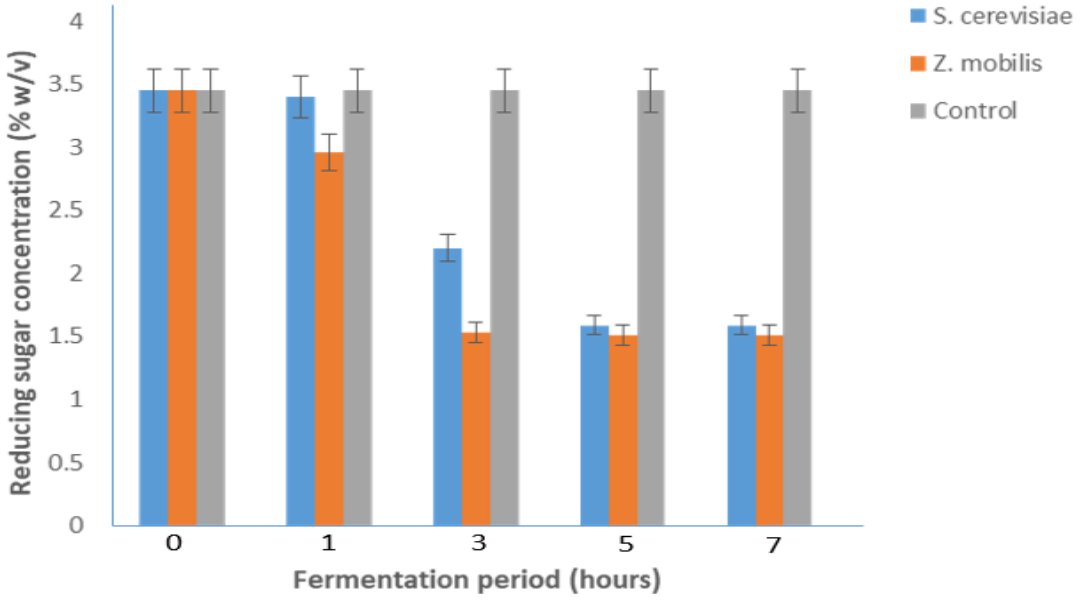


**Fig. 2. Changes in reducing sugar of the fermenting cassava peel samples**

**Table 2. Bioethanol produced from cassava peels**

| Sample fermented with | Initial volume (ml) | Final volume (ml) | Ethanol volume (ml) | Percentage yield (%) | Distillation range (°C) | Flash point (°C |
|---|---|---|---|---|---|---|
| *Z. mobilis* | 184 | 161 | 23 | 12 | 78-100 | 24 |
| *S. cerevisiae* | 150 | 105 | 45 | 30 | 78-100 | 24 |

The reduction in the pH value might be due to the consequent mixed fermentation process undergone by the microorganisms and their ability to produce organic acids (acetic acid, lactic acid, and succinic acid) in the fermentation medium, thus influencing the pH of the medium. Also, the anaerobic conditions created in the fermenters by the fermenting yeast can lower the pH. The pH condition may affect microbial survival, although most yeasts survive favorably at low pH [11]. According to Willaert and Nedovic [26], the authors reported that the pH of the surrounding medium can cause a change in the configuration and permeability of the cell membrane, thus reducing the rate of sugar fermenting enzymes or retarding metabolic pathway and microbial growth. Furthermore, authors also postulated that this trend might cause a reduction in the rate of ethanol production as fermentation time progresses. Competition for the available nutrients might also contribute to the growth of indigenous/inoculated organisms. A reduction in pH value of fermenting substrates has been reported to inhibit some pathogenic microorganisms [27,28].

The protein content of the fermented cassava peel showed a significant difference compared with the control. The yeast implicated in the fermentation of cassava peel suggests a possible prospect for protein improvement in various agro-industrial-based by-products. Inoculation of yeasts in the fermenting substrate that causes an increase in the protein content of the substrate could be due to the microbial ability in the synthesis of some extracellular enzymes (proteins), such as amylase, cellulase, protease, linamarase, and ligninase [29,30], into the fermentation medium in an attempt to utilize the waste as a source of carbon [22]. Also, increase in the protein contents of the inoculated cassava peel might be due to an increase in microbial biomass, ability of the microorganisms to synthesize amino acids, addition of crude proteins produced by bacteria isolate, and a combination of factors, such as carbon (iv) oxide, temperature and water, and cell proliferation in form of single-cell protein [31]. The difference in the protein content can be attributed to the type of fermenting substrates and fermenting microorganisms, as well as agro-ecological

conditions. The protein content of the fermented sample obtained from this study was lower compared to the higher protein content value of 21.5% of inoculated fermented cassava peels reported by Oboh [23]. Hence, it is evident that, due to the economic value of the enhanced nutritional value of fermented cassava peel by yeast isolates, it can serve as a source of protein and carbohydrate in livestock feeding.

The cell membrane of microorganisms is composed of organic, inorganic compounds, and some elements, such as lipid component, triglyceride. The resulting fermentation process showed an increase in the fat content of the inoculated fermented sample. The increase in the fat content of the fermented samples was in connection with the ability of yeast isolates to synthesize lipids during fermentation process [30]. High fat content might be due to microbial influence in the secretion of microbial oil, synthesis of lipase as well as the formation of protein-lipid or carbohydrate-lipid linkages. Also, increase in fat content corroborates earlier findings of Adeleke and Olaniyi [29], who reported an increase in the fat content of linamarase treated cassava peels.

Carbohydrates serve as a source of energy for microbial metabolism. A decrease in carbohydrate content of the inoculated fermented cassava peel compared to the control was observed. This can be attributed to the involvement of the yeast in the conversion of oligosaccharides into simple sugars, hydrolysis of starch to monomeric sugar form (glucose), thus serving as a source of carbon energy source for yeast proliferation and metabolism in a protein-rich medium [32]. It has been postulated that an increase in protein content of cassava peels can result in a decrease in the carbohydrate content; as during fermentation, sugar is used as a source of energy [23]. A decrease in carbohydrate content of the pretreated and naturally fermented cassava peels with increase in fermentation time has been reported [11]. Similarly, decomposition of cassava peels due to amylolytic activities of *Aspergillus niger* in the biotransformation of starchy component into simple reducing sugar under submerged state fermentation has been reported [33]. A reduction in the carbohydrate content of some fermented foods, such as cereal and legumes due to microbial activities during fermentation has been credited to the findings of Adegbehingbe*, et al.* [34]. A study by Okpako*, et al.* [35] reported a reduction in the carbohydrate content of 52.54% of cassava peel fermented with *A. niger* compared with the unfermented peels with a carbohydrate content of 72.50%.

During fermentation process, cassava peel inoculated with *S. cerevisiae* and *Z. mobilis* showed a reduction in value compared to the unfermented cassava peel (control). This revealed that the fermenting yeast can be potentially employed in the detoxification of toxic organic wastes to enhance maximum utilization for animal nutrition. Many researchers have reported a higher concentration of cyanogenic glycosides in the cassava peels compared to the whole cassava tubers, thus making it unfit as livestock feed [11,35,36]. Fermentation processes involving singularly or combined microorganisms reduced cyanide contents in cassava peels. The mixture of *A. niger* and *L. rhamnosus, S. cerevisiae* and *Lactobacillus* spp, and singular inoculation of cassava peel with *L. plantarum* in the reduction of cyanogenic glycosides in cassava peels have been reported[23,29,35], which conformed with the results obtained in this study.

Soaking of cassava and its products have been reported to reduce its cyanide content [37]. The cyanide content of the fermented cassava peel below the deleterious level of 30 mg/kg can be considered safe in terms of cyanide poisoning [36]. The reduction in the cyanide content has been attributed to the hydrolytic potential of linamarase enzyme, evaporation during drying, textural changes in plant tissues, maceration of the plant tissues in contact with linamarase by pressing out water during cassava processing, metabolic activities of inherent microorganisms with the ability to secrete extracellular enzymes (amylase, xylanase, and linamarase), increase in cell mass and formation of a hydrolytic complex bind force to the cyanide compound [11]. The reduction in cyanide content of fermented cassava waste treated with a mixed culture of *S. cerevisiae, L. delbruckii,* and *L. coryneformis* have been reported [36].

The reduction in the reducing sugar in the yeasts inoculated cassava peel can be attributed to the utilization of the sugar as a carbon source for the growth of the microorganisms (*S. cerevisiae and Z. mobilis*) and subsequent ethanol production. The reduction in the reducing sugar of co-cultured fermented corncobs with *A. niger* and *S. cerevisiae* has been reported [33]. The results obtain in this study were similar to the findings of Adegunloye and Udenze [38] who reported a

decline in the total reducing sugar of co-culture fermented cocoyam peel. The authors further stressed that the rapid fermentation of sugar to ethanol during the fermentation process by *S. cerevisiae* could continually lower the sugar concentration to prevent feedback inhibition of the amylolytic activity of certain molds at the onset of the fermentation.

Furthermore, inoculation of cell-free cassava peel hydrolysate with *S.cerevisiae* and *Z. mobilis* yielded utmost ethanol production after incubation for seven days, with sample inoculated with *S. cerevisiae* produced a high ethanol concentration of 30%. The ethanol production in the fermentation medium depends on the optimum conditions, such as pH, temperature, substrate concentration, and inoculum. The increase in bioethanol obtained might be due to the high amount of fermentable sugar in cassava peels, thus revealing ability of yeasts to produce certain extracellular enzymes in the hydrolysis of the substrate to produce simple reducing sugars needed for them to produce ethanol by fermentation. The ethanol concentrations of 2.3% and 3% obtained from cassava peel treated with enzymes and *S. cerevisiae* has been reported [39]. Studies have also identified the involvement of some bacteria/fungi in ethanol production, depending on their metabolic pathways driven by genes or special enzymes in the conversion of sugar to ethanol or other metabolites [25,40]. Fermentation of 500 g of cassava peels with *S. cerevisiae* with a resulting ethanol yield of 7.8% has been reported [24]. The maximum ethanol yield of 6% at 30$^{o}$C from banana fermented for 7 days with co-culture of *A. niger* and *S cerevisiae* had been reported [41]. Hence, the results obtained in this study were higher than the findings of Adegunloye and Udenze [38] who reported 6% maximum ethanol yield from cocoyam peel fermented with *A. niger* and *S cerevisiae.*

## 5. CONCLUSION

In conclusion, the improvement in the nutritional composition and reduction in the anti-nutritional content of the fermented cassava peel can be harnessed in the formulation of safe animal feed. The use of *S. cerevisiae* and *Z. mobilis* in the fermentation of cassava peel appreciably enhanced ethanol yield. Furthermore, the choice of cassava peel relies on the high carbohydrate content and high amount of metabolizable sugar needed by fermenting microorganisms. Hence, the high ethanol yield (45 mL) suggests that *S. cerevisiae* can be a suitable candidate in the fermentation of cassava peel for maximum bioethanol production. Therefore, this process can be harnessed for commercial ethanol production.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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