UMYU Scientifica, Vol. 2 NO. 3, September 2023, Pp 083 – 091
https://doi.org/10.56919/usci.2323.014
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Comparative Evaluation of Unpeeled Cassava (*Manihot esculenta*) and Tacca (*Tacca involucrata*) Tuber Flours as Substrates for Bioethanol Production.

Awodi, Philip Sule[1]*, Ujoh, Adole John[2], Adikwu, Peter[2] and Nwagu, Tochukwu Nwamaka[3].
[1]Department of Science Laboratory Technology, Benue State Polytechnic, Ugbokolo, Benue State, Nigeria.
[2]Department of Microbiology, Federal University of Health Sciences, Otukpo, Benue State, Nigeria.
[3]Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.

ABSTRACT
The ever-increasing demand for alternatives to fossil fuel due to its negative impacts on the environment and high prices have resulted in the search for feedstock for bioethanol production. Cassava is one of the major staple foods that is processed into various preservative forms in Nigeria, while tacca is a plant growing in the wild and is eaten by a few people during scarcity of food. Unpeeled cassava and tacca tubers were processed into flour. The flour was hydrolyzed using *Aspergillus niger* and *Saccharomyces cerevisiae,* separately. The bioethanol potentials of tuber flours were evaluated using a single-step process. Hydrolysis of 10g of cassava tuber flour separately by *Aspergillus niger* and *Saccharomyces cerevisiae* produced (g/100mL) 0.720 and 0.765 of sugar, respectively, while hydrolysis of 10g of tacca tuber flour separately by *Aspergillus niger* and *Saccharomyces cerevisiae* produced (g/100mL) 0.392 and 0.367 of sugar respectively. Evaluation of the effect of time during hydrolysis of cassava tuber flour for 24h by *Aspergillus niger* and *Saccharomyces cerevisiae* separately produced (g/100mL) 1.44 and 0.737 of sugar, while hydrolysis of tacca tuber flour for 24h produced (g/100mL) 0.768 and 0.493 of sugar. Evaluation of the effect of varying pH values during hydrolysis of the tuber flours revealed that pH 5.6 produced the highest concentration of sugar (0.240g/100mL) and (0.803g/100mL) when *Aspergillus niger* and *Saccharomyces cerevisiae* were separately used to hydrolyze cassava tuber flour. When *Aspergillus niger* and *Saccharomyces cerevisiae* were separately used to hydrolyze tacca tuber flour at pH 5.6, 0.626g and 0.436g of sugar was produced. Fermentation of cassava tuber flour by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisiae* for 48h produced 3.851%(w/v) bioethanol at 24h of fermentation, while fermentation of tacca tuber flour by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisiae* produced 3.236% (w/v) bioethanol at 48h. Cassava tuber flour produced a higher concentration of bioethanol than tacca tuber flour. These results have shown that tacca tuber is a potential feedstock for bioethanol production, hence exploitation of nonfood materials such as tacca tuber for bioprocesses can reduce the over -dependence on cassava tuber.

ARTICLE HISTORY
Received August 01, 2023.
Accepted September 20, 2023.
Published September 30, 2023.

KEYWORDS
cassava, fermentation, hydrolysis, single-step, tacca.

INTRODUCTION
Cassava is a plant that is cultivated because of the tuber which is consumed in various forms by every tribe in Nigeria. The tuber is also used extensively at the industrial level for production of various value–added products. Tacca (*Tacca involucrata)* is a plant that grows in the wild in Nigeria and it is eaten by a few people when food is scarce or during famine. The plant belongs to the family Taccaceae and the genus Tacca. Tacca grows in the North Central geopolitical zone in Nigeria, such as Benue, Kogi and Nassarawa States. It occurs in solitary forms on open fields or under the shade of trees or hilltops. In Plateau State, the tubers are a delicacy in Shendam, Langtang, and other lower Plateau, especially when other staple foods are scarce.Tacca is called "gbache" by Tiv tribe in Benue State, they use the tuber in preparing puddings and sauces when staple food is scarce. Tacca tubers are believed to be poisonous and contain a bitter principle, taccalin, and toxic saponins. Ruminants have been reported to have died as a result of consuming the peels of the tubers. Tacca starch's suitability for various uses in the food and pharmaceutical industries have been reported (Attama and Adikwu, 1999; Kunle *et al.*, 2003; Ofoefule *et al.*, 2004; Adebiyi *et al.*, 2011; Igbabul *et al.*, 2012; Jiang *et al.*, 2014). A study on tacca tuber storage under the ground was

Correspondence: Awodi, Philip Sule. Department of Science Laboratory Technology, Benue State Polytechnic, Ugbokolo, Benue State, Nigeria. ✉ philawosu@gmail.com. Phone Number: +234 706 289 7895.
How to cite: Awodi, P. S., Ujoh, J. A., Adikwu, P., & Nwagu, T. N. (2023). Comparative Evaluation of Unpeeled Cassava (*Manihot esculenta*) and Tacca (*Tacca involucrata*) Tuber Flours as Substrates for Bioethanol Production. *UMYU Scientifica*, 2(3), 83 – 91. https://doi.org/10.56919/usci.2323.014

reported by Raji and Ahemen (2016). Tacca plant still grows in the wild, despite the increasing discovery of its starch's importance in the food and pharmaceutical industries. Tacca tubers have some advantages over cassava and yam tubers: Tacca tuber can be stored under the ground for several years, the longer it remains in the soil as rain falls on it, the bigger the tuber becomes., tacca tuber does not decay (rot) in the soil before the vegetative part can grow, unlike yam tubers.Employment of tacca tubers in bioprocesses can create job and serves as another source of income for farmers of tacca,

Interest in the use of bioethanol as an alternative transport fuel has increased due to the advantages that it has over fossil fuel such as ; emission of less carbon dioxide, non-emission of toxic gases, has higher octane rating than gasoline, bioethanol production is eco-friendly and less energy is needed. Bioethanol is a renewable energy source which is produced from a variety of feed stocks such as corn and sugar cane (1st generation bioethanol), plant biomass such as agro wastes (2nd generation bioethanol), microalgae (3rd generation bioethanol) and by using genetically engineered microorganisms (4th generation bioethanol). Production of bioethanol from a previously-untapped biomass resources is referred to as a new generation. The global increment in population with high consumption of oil and natural gas has informed the exploration of sustainable and eco-friendly alternative sources of energy. Plant biomasses are targets for the supply of a sustainable energy resource due to their abundance and regenerative nature (Ebabhi *et al*., 2018). In order to make the alternative source cost effective, agro-wastes and non – food sources will be the most preferable plant biomass.

In recent years bioconversion of plant biomass such as water melon peels, cassava peels, mango seeds (Awodi *et al*, 2022), pawpaw seeds (Awodi *et al*, 2021) and pawpaw peels to bioethanol have received tremendous support from researchers (Bashir *et al*., 2021). The biofuel industries convert the starches into bioethanol by a batch fermentation process (Nugales et al, 2012), which comprises of three steps: (i) liquefaction of starch by starch hydrolyzing amylase to reduce viscosity of the starch (ii) saccharification process whereby the liquefied starch is hydrolyzed into fermentable sugar using glucoamylase, (iii) the fermentable sugar (glucose) is fermented into bioethanol by *saccharomyces cerevisiae* stains.

In industrial conversion of starch by batch fermentation process, the starch granules must be gelatinized and liquefied at high temperatures before saccharificution and fermentation (Matias et al 2015). This method which uses enzymes to liquefy and saccharify starches obtained from tubers and cereals have many disadvantages such as: (i) requirement of high energy in puts (ii) increase in production costs (Sharifa et al., 2009., Gohel & Suan 2012) out of the various methods such as SH&F and SS&F, SS&F is the most often preferred method (Nguyen et al 2014) due to the advantages SS&F has over other methods which include, reduction of cost because less equipment and fermentation time are needed.

There is lack of information on bioconversion of tacca tuber into bioethanol in available literatures. Hydrolysis & fermentation of tacca tuber flour was compared with cassava tuber flour and instead of the high-temperature cooking method of producing bioethanol, a single-step bioethanol production process was adopted in this research. The present research, therefore, aims to produce and compare the sugar and bioethanol yield potentials of unpeeled cassava and tacca tuber flours using a single–step process.

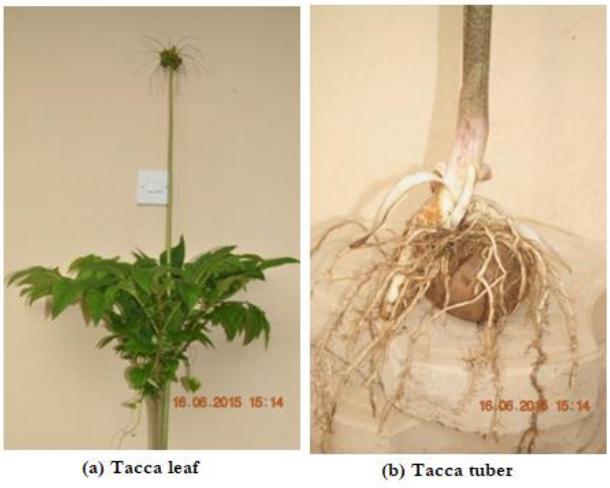

Plate 1. *Tacca involucrata* plant

MATERIALS AND METHODS

Organism

The *A.niger* used was obtained from the Department of Microbiology, University of Nigeria, Nsukka. The yeast isolate used in this research was isolated from "burukutu", a locally fermented alcoholic beverage which is often produced from *Sorghum vulgare* in Nigeria. The yeast was identified (*Saccharomyces cerevisiae KM103041)* at IITA, Ibadan. All chemicals used were of analytical grade

Preparation of plant materials (cassava and tacca tubers)

Fresh cassava tubers were bought from a cassava processing industry in Okete, Ohimini LGA of Benue State, Nigeria, while tacca tubers were sourced from forest in Otukpo and Ugbokolo environments in Benue State, Nigeria. The tubers were washed with clean water to remove soil from the tubers, thereafter they were sliced and dried under the sun for 4weeks. The dried cassava and tacca tubers were ground separately into powder in a commercial grinding machine in Otukpo main market, the flour was filtered. The filtrate was stored in a polythene bag until needed for experiments.

Screening of *Aspergilus niger* and Saccharomyces cerevisiae for amylase production.

Both *A.niger* and *S. cerevisiae* were screened for amylase production. The isolates were cultured in a starch based medium (nutrient agar and 5% starch). The plates were incubated for 24h and 72h for *S.cerevisiae* and *A.niger* respectively, at 30°C . The fungal growth and *S. cerevisiae* cells were flooded with iodine solution for determination of amylase production. The isolates (*A.nger* and *S, cerevisiae*) with the widest zone of hydrolysis were chosen to be used in the cassava and tacca tuber flours hydrolysed.

Screening of *Saccharomyces cerevisiae* for alcoholic tolerance.

S. cerevisiae was initially cultivated in PDA for 24 h, after appreciable growth, the isolates were transferred into 100mL of various concentrations (10, 20, 30, 40, 50)% of 95% ethanol for 24h. The isolates were subcultured into PDA again. The isolate that grew faster and abundantly in 30% of the 95% ethanol concentration was chosen.

Analytical methods

i. **Determination of fermentable sugar concentration:** The fermentable sugar concentrations produced from cassava and tacca tuber flour using *A.niger* & *S.cerevisiae* was determined by DNS method (Miller,1959).
ii. **Bioethanol concentration:** Bioethanol concentrations produced from cassava and tacca tuber flours was determined using HPLC (Cecil instrument). U.K, at Sheda Science Complex, Abuja, Nigeria.
iii. **Temperature:** The temperature of the fermenting broth was determined using a digital thermometer (mextech multi – thermometer model, China). The thermometer was inserted into the fermenting flour and the temperature was noted.
iv. **pH:** The pH value of the fermenting broth was measured by using a digital pH meter (HI 96107), Hanna Instruments model. China. The pH meter was inserted into the fermenting flour and the pH value was noted.

Statistical analysis

The data were expressed as mean standard deviation (S.D). The data were statistically analyzed using one way analysis of variance (ANOVA). The values were considered significant at $P<0.05$.

Medium for hydrolysis of cassava and tacca tuber flours for sugar production

The media used for hydrolysis of cassava and tacca tuber flours using *A. niger* consisted of varied quantities (g/100mL) : 2-10 of cassava and tacca tuber flours separately in 250mL conical flasks, peptone,1.5 and water,100mL for verification of the effect of substrate (cassava and tacca tuber flour) concentrations on sugar production. For verification of the effect of time on sugar production from the substrates (cassava and tacca tuber flour), the medium consisted of 10g of cassava and tacca tuber flour per 100mL of distilled water and peptone, 1.5g separately in 250mL conical flasks. The medium used for verification of the effect of pH on sugar production consisted of 10g of cassava and tacca tuber flours in 100mL of citrate phosphate buffer of various pH (3.6, 4.6 & 5.6) values in 250mL conical flasks separately. The medium used for verification of the effect of inoculum (*A.niger*) on sugar production consisted of 10g of cassava and tacca tuber flour in 100mL of citrate phosphate buffer , pH (5.6) in 250mL conical flasks separately. All the media were sterilized in an autoclave at 121°C at 15psi for 15mins. *Saccharomyces cerevisiae* was also used to hydrolyze the substrates (cassava and tacca tuber flour) for sugar production.

Fermentation medium

The media used for fermentation of cassava and tacca tuber flour using a mixed culture of *Aspergillus niger and Saccharomyces cerevisiae* consisted of (g/100mL)., peptone, 1.5., substrate,10., citrate phosphate buffer (pH 5.6) 100mL. All the media were sterilized in an autoclave at 121°C at 15psi for 15mins.

Optimization of hydrolysis processes

Evaluation of the effect of substrate (cassava and tacca tuber flour) concentrations on sugar production using A.niger

Different quantities (2-10g) of cassava tuber flour were dispensed into 250ml conical flasks containing 100mL each of the sterilized medium which consisted of distilled water, 100 mL and peptone, 1.5g. *A. niger* spore ($3x10^5$ SFU/mL) was inoculated into the medium. The substrate was hydrolyzed for 24 hours at 30^0c in an incubator. At the end of hydrolysis, 10mL was withdrawn from the broth and centrifuged at 3000 rpm for 5 mins. The supernatant was collected and used for sugar determination using Miller (1959) method. For comparative purposes, tacca tuber flour was used as substrate for evaluation of the effect of substrate concentration on sugar production also.

Evaluation of the effect of time on sugar production from cassava and tacca tuber flour using *A. niger.*

The hydrolysis medium contains 1.5g of peptone in 100mL of distilled water. The medium was sterilized at 121^0c for 15 mins. After cooling, 10g of the substrate (cassava tuber flour) was added, *A. niger* ($3x10^5$ SFU/mL) was inoculated into the medium and incubated at 30^0c for 48 hours. Samples (10mL) was withdrawn from the broth at 12 hour intervals and centrifuged at 3000rpm for 5mins. The supernatant (1mL) was used for sugar analysis using Miller (1959) method. Tacca was subjected to hydrolysis for sugar production as described above using *A. niger.*

Evaluation of the effect of pH on sugar production from cassava and tacca tuber flours using *A. niger.*

Citrate phosphate buffer of various pH values (3.6, 4.6 and 5.6) were prepared and 100mL of each was dispensed into different 250mL conical flasks in duplicates, 1.5g of peptone was added. The medium was sterilized. After cooling 10g of cassava tuber flour was added and *A. niger* spores ($3x10^5$ SFU/mL) was inoculated into the medium. The medium was incubated at 30^0c for 24 hours. After hydrolysis, 10mL was withdrawn and centrifuged at 3000 rpm for 5 mins. The supernatant (1mL) was used for sugar determination using Miller (1959) method. Tacca tuber flour was also hydrolyzed as described above.

Evaluation of the effect of inoculum (*A. niger*) size on sugar production from cassava and tacca tuber flour.

The medium consisted of 100mL of citrate phosphate buffer (PH 5.6) and peptone, 1.5g, the medium was sterilized as described previously. After cooling, 10g of cassava tuber flour was added and *A. niger* ($3x\ 10^5$ SFU/mL) was inoculated into it and hydrolyzed for 24 hours at 30^0c, 10mL was pipetted from the medium and centrifuged at 3000 rpm for 5 mins. Sample (1mL) of the supernatant was used for sugar determination. Tacca tuber flour was also used as substrate and hydrolyzed as described above. Sugar concentration was assessed using DNS method of Miller (1959).

Evaluation of the effect of substrate (cassava and tacca tuber flour) concentrations on sugar production using *S.cerevisiae*

Different quantities (2-10g) of cassava tuber flour were dispensed into 250ml conical flasks containing 100mL of previously sterilized medium which consisted of 100mL of distilled water and 1.5g of peptone. *S.cerevisiae* ($5x10^5$ CFU/mL) was inoculated into the medium. The substrate was hydrolyzed for 24 hours at30^0c in an incubator. At the end of hydrolysis, 10mL was taken and centrifuged at 3000 rpm for 5 mins. The supernatant (1ml) was collected and used for sugar determination using Miller (1959) method. Tacca tuber flour was also used as substrate for evaluation of the effect of substrate concentration on sugar production.

Evaluation of the effect of time on sugar production from cassava and tacca tuber flour using *S.cerevisiae*

The hydrolysis medium contains 1.5g of peptone in 100mL of distilled water. The medium was sterilized at 121^0c for 15 mins. After cooling, 10g of the substrate was added. *S.cerevisiae* ($5x10^5$ CFU/mL) was inoculated into medium and incubated at 30^0c for 48 hours. Samples (10mL) was withdrawn from the broth at 12h intervals and centrifuged at 3000rpm for 5mins. The supernatant was used for sugar analysis using Miller (1959) method. Tacca was subjected to hydrolysis for sugar production as described above also.

Evaluation of the effect of pH on sugar production from cassava and tacca tuber flour using *S. cerevisiae*

Citrate phosphate buffer of various pH values (3.6, 4.6 and 5.6) were prepared and 100mL of each was dispensed into different 250ml conical flasks in duplicates, 1.5g of peptone was added into each. The medium was sterilized. After cooling, 10g of cassava tuber flour was added, S.cerevisiae ($5x10^5$ CFU/mL) was inoculated into the medium. The medium was incubated at 30^0c for 24 h. After hydrolysis, 10mL was withdrawn and centrifuged at 3000 rpm for 5 mins. The supernatant (1mL) was used for sugar determination using Miller (1959) method. Tacca tuber flour was also hydrolyzed as described above.

Evaluation of the effect of inoculum (*S. cerevisiae*) size on sugar production from cassava and tacca tuber flour.

The medium consisted of 100mL of citrate phosphate buffer (pH 5.6) and peptone, 1.5g, the medium was sterilized as described previously. After cooling, 10g of cassava tuber flour was added and *S.cerevisiae* ($5x\ 10^5$ CFU/mL) was inoculated into it and hydrolyzed for 24 h at30^0c. Thereafter, 10mL was pipetted from the medium and centrifuged at 3000 rpm for 5 mins. Sample (1mL) of the supernatant was used for sugar determination. Tacca tuber flour was also used as substrate and hydrolyzed as described above. Sugar concentration was assessed using DNS method of Miller (1959).

Fermentation of cassava and tacca tuber flour into bioethanol using a mixed culture of A. niger and S. cerevisiae.

A. niger spores ($3x10^5$ SFU/mL) and *S. cerevisiae* ($5x10^5$ CFU/mL) was inoculated into previously sterilized fermentation medium (batch fermentation) which consisted of 10g of substrate (cassava), 100mL of citrate phosphate buffer pH (5.6) and 1.5g of peptone. The medium was fermented for 48h at 30^0c. Samples (10ml) was withdrawn at 12h intervals and centrifuged at 3000rpm for 5 mins. The supernatant was used for bioethanol determination using HPLC.

RESULTS

Evaluation of the effect of substrate concentration on sugar production from cassava and tacca tuber flour.

The effect of substrate concentration on sugar production (Fig.1a) showed that as substrate increases from 2 to 10g/100mL there was gradual increase in the sugar generated(g/100mL) from 0.222 to 0.720 and from 0.0850 to 0.392 when *A. niger* was used to hydrolyze cassava and tacca tuber flour. When *S. cerevisiae* was used to hydrolyze cassava and tacca tuber flour (Fig. 1b) the sugar produced (g/100mL) increased from 0.170 to 0.765 and from 0.0677 to 0.367 respectively.

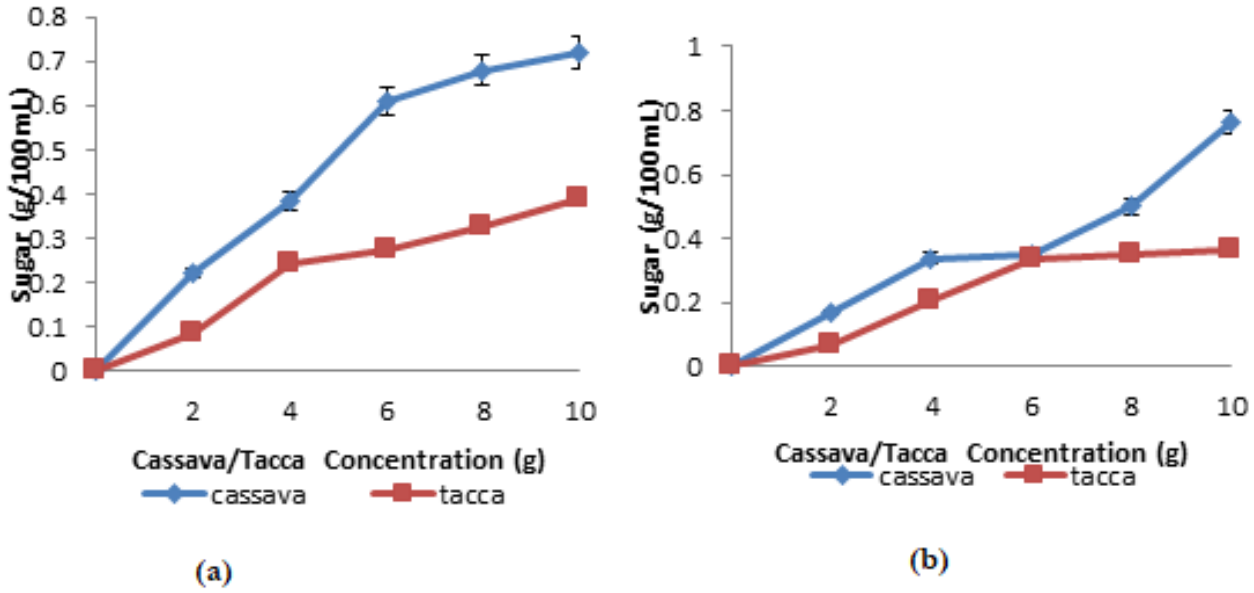

Figure 1: Evaluation of the effect of cassava and tacca tuber flour concentrations on sugar production (a) Using *Aspergillus niger* . (b) using *Saccharomyces cerevisiae.*

Evaluation of the effect of time on sugar production from cassava and tacca tuber flour.

The time course of sugar production from cassava and tacca tuber flour using *A. niger*, showed increase in the sugar production from 0.720g/100mL to 1.44g/100mL between 12h and 24h (Fig.2a.). Beyond 24h, the sugar generated began to decrease from 1.44g/100mL to 0.998 (g/100mL) but when tacca tuber flour was hydrolyzed by *A. niger*, the sugar generated increased from 0.392g/100mL to 0.965 (g/100mL) between 12h to 36h. At 48h, there was a decrease in the sugar concentration. Hydrolysis of cassava tuber flour using *S. cerevisiae* (Fig. 2b) showed that the highest amount of sugar was generated at 12h. Beyond 12h, the sugar generated began to decrease from 0.737g/100mL to 0.549 (g/100mL), while hydrolysis of tacca using *S. cerevisiae* revealed that the highest amount of sugar was produced at 48h (0.547g/100mL).

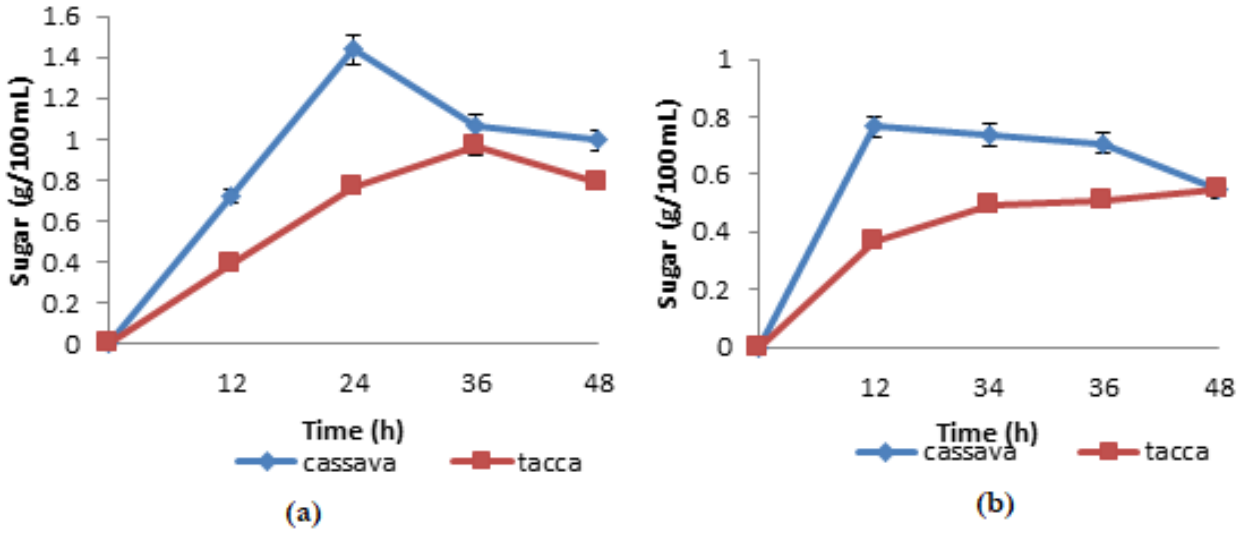

Figure. 2: Evaluation of the effect of time on sugar production from cassava and tacca tuber flour (a) using *Aspergillus niger*. (b) using *Saccharomyces cerevisiae.*

Evaluation of the effect of pH on sugar production from cassava and tacca tuber flour.

The effect of pH (3.6, 4.6, 5.6) on sugar production from cassava tuber flour when *A. niger* was used to hydrolyze the flour (Fig.3a) revealed that pH 5.6 generated the highest amount of sugar (0.240g/100mL), similarly, when tacca was hydrolyzed by A. niger, pH 5.6 generated the highest amount of sugar (0.626g/100mL).

Hydrolysis of cassava tuber flour using *S.cerevisiae* showed that pH 4.6 generated the highest amount of sugar

0.912g/100mL (Fig3b), while pH 3.6 generated the least amount of sugar from cassava tuber flour, (0.246). Hydrolysis of tacca tuber flour using *S. cerevisiae* showed that pH 4.6 also generated the highest amount of sugar (0.947g/100mL|), while pH 5.6 generated the least sugar concentration.

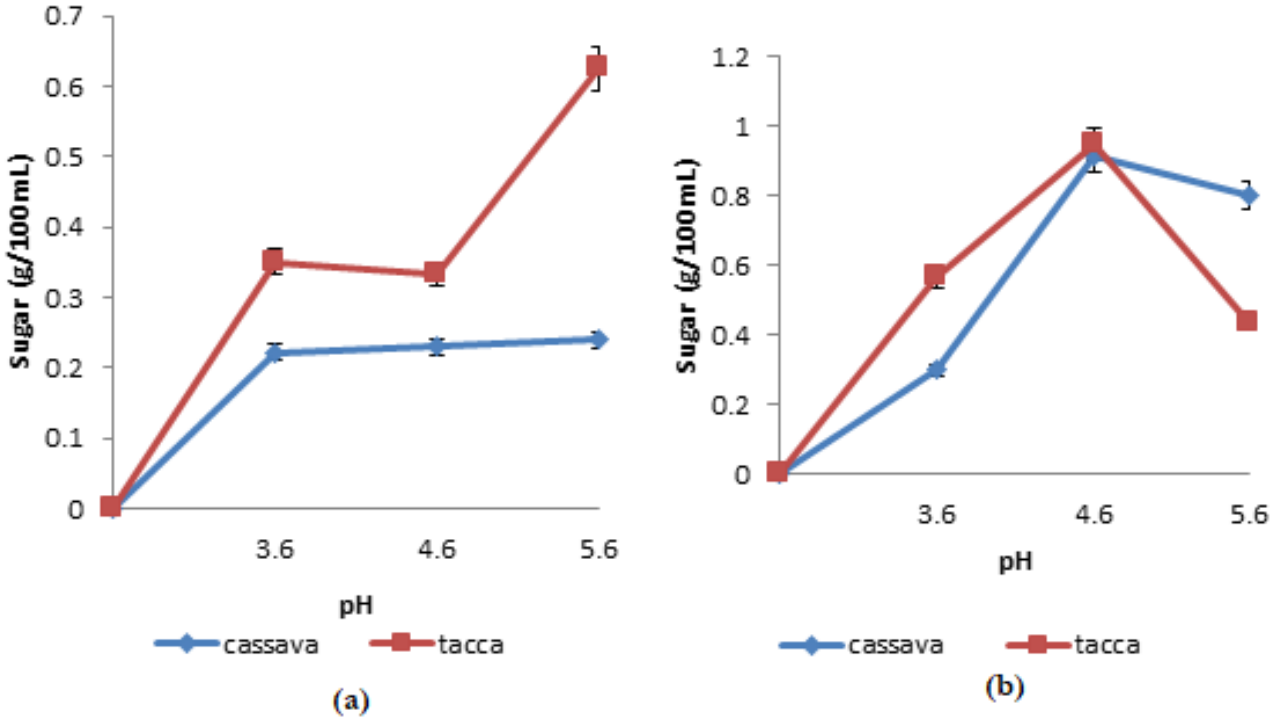

Figure 3: Evaluation of the effect of pH on sugar production from cassava and tacca tuber flour (a) using *Aspergillus niger.* (b) using *Saccharomyces cerevisiae.*

Evaluation of the effect of inocula concentrations on sugar production from cassava and tacca tuber flour.

Evaluation of the effect of inocula concentrations were assessed (Fig. 4). Increase in sugar production from 0.39g/100mL to 0.433g/100mL was seen as inocula concentrations increased when *A. niger* was used to hydrolyze cassava tuber flour (Fig. 4a), beyond 2 x 10^5 (SFU/mL) of *A. niger* concentration, a decrease in sugar production from 0.403g/100mL to 0.401(g/100mL) was observed. Similarly, 2x10^5 (SFU/mL) of *A. niger* generated more sugar (0.502g/100mL) when tacca tuber was hydrolyzed, thereafter a decrease in sugar production from 0.469g/100mL to 0.431g/100mL was observed (Fig.4a).

Hydrolysis of cassava tuber flour using *S.cerevisiae* (Fig.4b) revealed that 4mL (5x10^5 CFU/mL) generated the highest concentration of sugar (0.777g/100mL), 4mL (5x10^5CFU/mL) of *S. cerevisiae* also generated the highest concentration of sugar (0.530g/100mL) from tacca tuber flour.

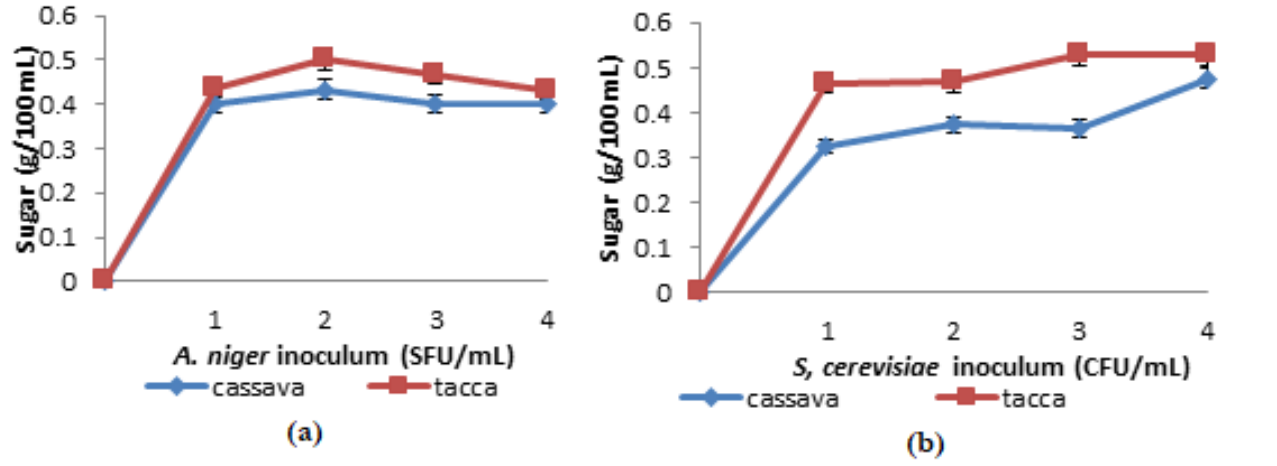

Figure 4: Evaluation of the effect of inoculum concentrations on sugar production from cassava and tacca tuber flour. (a) using *Aspergillus niger.* (b) using *Saccharomyces cerevisiae*

Fermentation of cassava and tacca tuber flour into bioethanol

The optimum parameters previously assessed for production of fermentable sugar from the substrates were used to ferment the substrates (cassava and tacca tuber flour) into bioethanol using a mixed culture of *A. niger and S. cerevisiae* (Tables 1 & 2).

The bioethanol concentration was determined using HPLC. The highest concentration of bioethanol was produced at 24h when cassava tuber flour was fermented into bioethanol, contrary to the fermentation of tacca tuber flour that produced highest concentration of bioethanol at the 48h. This result suggests that fermentation of tacca tuber flour takes a longer time than fermentation of cassava tuber flour.

Table 1: Fermentation of Cassava tuber flour into bioethanol using a single – step process

Time (h)	Temperature (°C)	pH	Bioethanol% (v/v)
0	31.3	4.5	0.00
12	31.3	4.5	2.309
24	32.7	4.1	3.851
36	34.1	3.6	2.770
48	31.1	3.1	0.541

Table 2: Fermentation of tacca tuber flour into bioethanol using a single – step process

Time (h)	Temperature (°C)	pH	Bioethanol % (v/v)
0	31.3	4.6	0.00
12	31.3	4.5	2.19
24	32.6	4.0	2.61
36	33.9	3.8	2.94
48	30.9	3.8	3.23

DISCUSSION

The effect of substrate concentration on sugar production showed that the maximum sugar concentration was obtained with 10g/100mL of both cassava and tacca tuber flour when *A. niger and S. cerevisiae* were separately used to hydrolyze the substrates. Generation of the highest amount of glucose with 10% w/v of the substrate agrees with Sanette and Tundo (2013)'s report while working on unpeeled cassava tuber. These results indicate that tacca and cassava tuber flour are susceptible to hydrolysis by fungi, value added-products like glucose and bioethanol can be produced by fermentation method from both substrates. Hydrolysis of raw unpeeled cassava tuber flour by *A. niger* generated more fermentable sugar (0.720g/100mL)w/v than tacca tuber flour (0.392g/100mL) w/v, the result indicates that the fermentable sugar produced from raw cassava tuber flour was significantly higher ($P<0.05$) compared to fermentable sugar produced from raw tacca tuber flour. Hydrolysis of raw cassava tuber flour by *S. cerevisiae* also generated more fermentable sugar (0.765g) w/v than raw tacca tuber flour (0.367g) w/v.

The time course of fermentable sugar production during hydrolysis was evaluated over a time range of 12h to 48h. Maximum sugar (1.44g) w/v concentration was obtained at 24h when A. niger was used to hydrolyze cassava tuber flour, while maximum fermentable sugar was obtained at 36h when *A. niger* was used to hydrolyze tacca tuber flour (0.965g) w/v. There was significant difference ($P<0.05$) between the sugar concentrations produced from cassava tuber flour and tacca tuber flour when A. niger was used to hydrolyze the substrates.

Saccharification of cassava and tacca tuber flour by *S. cerevisiae* revealed that the maximum fermentable sugar was generated at 36h and 48h respectively. The present results shows that the rate of hydrolysis of cassava tuber flour using *S.cerevisiae* was faster than tacca tuber flour. There was also significant difference ($P<0.05$) in the concentrations of sugar generated from both substrates.

Evaluation of the effect of varying pH (3.6, 4.6, 5.6) values during optimization of saccharification process, revealed that pH 5.6 generated the maximum sugar concentration when *A. niger* was used to hydrolyze cassava and tacca tuber flour (0.240g and 0.626g)w/v, respectively. This result shows that the introduction of citrate phosphate buffer into the medium has enhanced the saccharifying activity of A. niger, hence more sugar was generated from tacca tuber flour than cassava tuber flour, which is contrary to the previous results obtained when the effects of substrate concentration and time of fermentation were evaluated in this study. The fermentable sugar generated from tacca tuber flour was significantly higher ($P<0.05$) than the sugar generated from cassava tuber flour.

Hydrolysis of cassava and tacca tuber flour using *S cerevisiae.* showed that the maximum fermentable sugar was generated at pH4.6 (0.912 and 0.946) respectively. Cassava tuber generated more sugar than tacca tuber. The production of maximum sugar at pH 4.6 when *S. cerevisiae* was used agrees with Morakot et al (2021) while working with cassava starch for bioethanol production, they reported that pH 4.5 gave the highest ethanol production using *S.cerevisiae.* Optimal pH values for yeast growth could vary from 4.0 to 6.0, pH is considered as an important factor for survival and growth of yeasts. It affects the permeability of the cell membrane and on the enzymes that are active in degrading the substrate (Arroyo-Lope etal, 2009). The maximum sugar concentration obtained in this study at pH 4.6 is contrary to Sanette & Tando (2013)'s report, that pH had significant effect on the glucose yield with the highest yield of 0.4+0.001g obtained at pH 6.

CONCLUSION

The comparative evaluation of cassava tuber flour and tacca tuber flour for fermentable sugar production in this study has shown that cassava tuber flour produced more

sugar (0.720g/100mL) than tacca tuber flour (0.392g/100mL) when *Aspergillus niger* was used to hydrolyze both flours, similarly, when *Saccharomyces cerevisiae* was used to hydrolyze both flours, cassava tuber flour generated 0.599g/100mL of sugar while tacca flour generated 0.367g/100mL of sugar. Fermentation of cassava tuber flour by mixed culture of *A. niger* and *S. cerevisiae* revealed that cassava tuber flour generated higher concentration (3.851%) w/v of bioethanol than tacca tuber flour (3.236 %) w/v. These results have shown that tacca tuber is a potential feedstock substitute to cassava tuber for bioprocesses.

REFERENCES

Adebiyi, A.B., Omojala, M.O., Afolayan, M.O., Zaku, S.G. &Olalekan, D. (2011). Tacca starch citrate-a potential pharmaceutical excipient. *Int. J. Pharm. Res. Rev.*, **3** (8): 1-7.

Arroyo-Lope, F.N., Orlic, S., Querol, A., & Barrio, E. (2009). Effect of temperature, pH and sugar concentration on the growth parameters of S. cerevisiae, S. Kudriavzevil and their interspecific hybrid. *International Journal of Food Microbiology* 131 (2-3): 120 – 127). **[Crossref]**

Attama, A.A. and Adikwu, M.U. (1999). Bioadhesive delivery of hydrochlorothiazide using tacca starch/SCMC and tacca starch/Carbopols 940 and 941 admixtures.*Bolletinofarmaceutico***138** (7): 343 -350. PMID: 10597656.

Awodi, P.S., Nwagu. T.N., Tivkaa, J., Ella, A.B. & Ogbonna, J. (2021). Simultaneous Saccharification and Fermentation of pawpaw (*Carica papaya*) seeds for bioethanol production. *Vegetos,* 34 (3): 671 – 677. **[Crossref]**

Awodi, P.S., Ogbonna, J.C. Nwagu, T.N. (2022). Bioconversion of mango (*Mangifera indica) seed kernel starch into bioethanol using various fermentation techniques. Heliyon,8(6): e09707.* **[Crossref]**.

Bashir, I.O., Mahmud, Y.I., Bashir, M., Ahmad, A. & Ahmed, F.U. (2021). Production of Bioethanol by Co-Culture of Aspergillus niger and Saccharomyces cerevisiae using Watermelon Peels as Substrate. *Traektoriâ Nauki= Path of Science*, *7*(10), 6001-6011. **[Crossref]**

Ebabhi, A. M., Adekunle, A. A., & Adeogun, O. O. (2018). Potential of some tuber peels in bioethanol production using Candida tropicalis. *Nigerian Journal of Basic and Applied Sciences*, *26*(2), 17-22. **[Crossref]**.

Gohel, V., Duan, G. (2012). No-cooked process for ethanol production using Indian broken rice and pearl millet. *International Journal of Microbiology*, Volume 2012, Article ID 680232, **[Crossref]**.

Igbabul, B.D., Ariahu, C.C. and Umeh, E.U. (2012). Moisture desorption isotherms of African arrowroot lily (*Tacca involucrata*) tuber mash as influenced by blanching and natural fermentation. *Journal of Food Technology* **10** (1): 8 - 16.

Jiang, J.H., Yang, H.M., Wang, Y.L. and Chen, Y.G. (2014). Phytochemical and pharmacological studies of the genus Tacca ®: A review. *Tropical Journal of Pharmaceutical Research* **13** (4): 635 – 648. **[Crossref]**

Kunle, O.O., Ibrahim, Y.E., Emeje, M.O., Shaba, S. and Kunle, Y. (2003). Physicochemical properties of *Tacca involucrata* starch. *Starch – starke* **55** (7): 319 – 325. **[Crossref]**

Matias, J., Encinar, J.M., Gonzalez, J., Gonzalez, J.F. (2015). Optimization of ethanol fermentation of Jerusalem artichoke tuber juice using simple technology for a decentralized and sustainable ethanol production. *Energy for Sustainable Development. 25:34-39.* **[Crossref]**

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*, *31*(3), 426-428. **[Crossref]**

Morakot, K., Kwanruthai, M., Jatuporn, S., Krongchan, R., & Saethawat, C. (2021). Single-step ethanol production from raw cassava starch using combination of raw starch hydrolysis and fermentation,, scale up from 5-L laboratory and 200-L pilot plant to 300-L industrial fermenters. *Biotechnology for Biofuels*, *14*(1), 1-15. **[Crossref]**.

Naguleswaran, S., Li, J., Vasanthan, T., Bressler, D., & Hoover, R. (2012). Amylolysis of large and small granules of native triticale, wheat and corn starches using a mixture of α-amylase and glucoamylase. *Carbohydrate polymers*, *88*(3), 864-874. **[Crossref]**

Nguyen, C. N., Le, T. M., & Chu-Ky, S. (2014). Pilot scale simultaneous saccharification and fermentation at very high gravity of cassava flour for ethanol production. *Industrial crops and products*, *56*, 160-165. **[Crossref]**

Ofoefule, S.I., Osuji, A.C. & Okorie, O. (2004). Effects of physical and chemical modifications on the disintegrant and dissolution properties of *Tacca involucrata* starch. *Bio-Research*, *2*(1), 97-102. **[Crossref]**

Raji, A.O. & Ahemen, S.A. (2016). Underground storage of *Tacca involucrata* tubers. *Research Journal of Applied Sciences, Engineering and Technology*, *12*(2), 142-146.

Sanette, M., & Tando, Y.C.N. (2013). Cassava as feed stock for ethanol production in South Africa. *Africa Journal of Biotechnology*, vol. 12 (31): PP 4975-4983. **[Crossref]**

Shariffa, Y.N., Karim, A.A., Fazilah, A., & Zaidul, I.S.M. (2009). Enzymatic hydrolysis of granular native and mildly-heat treated tapioca and sweet potato starches at sub-gelatinization temperature. *Food hydrocolloids*, *23*(2), 434-440. **[Crossref]**