Original Research Article

TEST FOR REDUCING SUGAR CONTENT OF CASSAVA WASTE (PEELS) DURING SOLID STATE FERMENTATION FOR TREATED AND UNTREATED SAMPLE.

ABSTRACT

In Nigeria, cassava production is well-developed as an organized agricultural crop. It has well-established multiplication and processing techniques for food products and cattle feed. There are more than 40 cassava varieties in use. Reducing Sugar Content of Cassava waste (Peels) during solid state fermentation for Untreated Samples showed that the mixed culture of *Aspergillusniger* and *Aspergillusflavus* has the highest reducing sugar content, 32, 28 and 27 for cassava waste TMS/98/0581, TME/98/419 and TMS/98/30572 respectively at day 9 and single culture of *Aspergillusniger* having the lowest reducing sugar content, 4, 7, and 6 for cassava waste TMS/98/0581, TME/98/419 and TMS/98/30572 respectively at day 9 and single culture of *Aspergillusniger* having the lowest reducing sugar content, 4, 7 and 6 for cassava waste TMS/98/0581, TME/98/419 and TMS/98/30572 respectively at day 3. Also reducing sugar content of cassava waste (Peels) during Solid state fermentation for Treated sample showed that the mixed culture of *Aspergillusniger* and *Aspergillusflavus* has the highest reducing sugar content, 60, 54 and 50 for cassava waste TMS/98/0581, TME/98/419 and TMS/98/30572 respectively at day 6 and single culture of *Aspergillusniger* having the lowest reducing sugar content, 18, 16 and 14 for cassava waste TMS/98/0581, TME/98/419 and TMS/98/30572 respectively at day 3. Statistical analysis using paired t-test showed that there was significant difference between the mean valves obtained from treated cassava waste and untreated cassava at $P < 0.05$. the feasibility of bioconversion of cassava waste into value added products was demonstrated in this study.

KEY WORDS: Cassava, reducing sugar, starch, *Aspergillus niger, Aspergillus flavus*

1. INTRODUCTION

Cassava (*Manihot esculenta Crantz*) also known as tapioca, is a native of tropical South America and is a major root crop grown for its starchy roots. It is a cheap source of carbohydrate in the humid tropics (Okigbo and Anyaegbu, 2021). Cassava processing contributes significantly to environmental pollution and aesthetic nuisance. The major waste of cassava processing in Nigeria is cassava peels. Efficient and controlled biodegradation of this material by fungi or bacteria leads to a number of processes of great economic importance (Robinson and Nigam, 2003).

Since these peels could make up to 20-35% of the wet weight of the roots, they constitute an important potential resource if properly harnessed biotechnologically (Obadina *et al.*, 2006). Fermentation has been identified as one of the less expensive means of detoxification and increase the porosity of the lignocellulosic material and also enhance sugar production by reducing the possibility of loss of carbohydrate. (Gélinas and Barrette, 2009).

About 10 million tonnes of cassava are processed for garri annually in Nigeria alone (Okafor *et al.*, 2008). In the processing of cassava fermented products, the roots are normally peeled to rid them of two outer coverings: a thin brown outer covering and a thicker leathery parenchymatous inner covering. These peels are regarded as wastes and are usually discarded and allowed to rot. With hand peeling, the peels can contribute 20 – 35% of the total weight of the tuber (Obadina *et al.*, 2006). The waste generated at present pose a disposal problem and would even create more problems in the future with increased industrial production of cassava products such as cassava flour and dried cassava fufu.

Starch is a promising substrate for the production of glucose, fuels and single cell protein (SCP). Various amylolytic fungi have been used for the production of SCP and amylase from starchy materials in submerged agitated culture (Soccol and Vandenberghe, 2003). Sevral workers, however, have turned their attention to biodegradation of starch using solid state fermentation because this method has been found to be the more appropriate system than submerged fermentation for protein enrichment and amylase production from starchy materials (Pandey and Soccol, 2001).

Despite its importance, cassava is mostly grown by small farmers on small plots of land. Urban consumers and factories obtain their cassava from rural areas where it is grown. Cassava is usually processed immediately after it is taken from the ground because it is highly perishable. Spoiling starts within 48 to 72 hours after harvest. A mature cassava root (hereafter referred to as 'root') may range in length from 15 to 100 cm and weigh 0.5 to 2.5 kg. Circular in cross-section, it is usually fattest at the proximal end and tapers slightly towards the distal portion. It is connected to the stem by a short woody neck and ends in a tail similar to a regular fibrous root.

Processing of cassava is generally considered to contribute significantly to environmental pollution and aesthetic nuisance. The two major wastes of cassava processing in Nigeria are cassava sievates (a product

from garri processing), and cassava offal (wastes from fufu production). About 10 million tonnes of cassava are processed for garri annually in Nigeria alone (Okafor *et al.*, 2008). In the processing of cassava fermented products, the roots are normally peeled to rid them of two outer coverings: a thin brown outer covering, and a thicker leathery parenchymatous inner covering. These peels are regarded as wastes and are usually discarded and allowed to rot. The peels can constitute 20 - 35% of the total weight of the tuber, with hand peeling (Okigbo and Anyaegbu, 2021). The wastes generated at present pose a disposal problem and would even be more problematic in the future with increased industrial production of cassava products such as cassava flour and dried cassava fufu. Products of fermentation of cassava peels from such heaps include foul odour and sometimes poisonous and polluted air, which when inhaled by man or animals may result into infection and diseases that may take a long time to manifest. In the same vein, vegetation and soil around the heaps of cassava peels are rendered unproductive and devastated due to biological and chemical reactions taking place between the continuously fermenting peels, soil and the surrounding vegetation. Since these peels could make up to 10% of the wet weight of the roots, they constitute an important potential resource if properly harnessed biotechnologically (Obadina *et al.*, 2006). One of the less expensive means of detoxification and increase of the protein quality of cassava has been identified by fermentation. The use of microorganisms to convert carbohydrates, lignocelluloses and other Industrial wastes into foodstuffs rich in protein is possible due to the following characteristics of microorganisms: ability for a very fast growth rate, can be easily modified genetically for growth on a particular substrate under particular cultural conditions, high protein content varying from 35 to 60%, ability to grow in slurry or on solids and their nutritional values are as good as other conventional foods rich in protein. Bayitse (2013) reported that cassava liquid residue contains minerals (nitrogen, carbon, phosphorus, potassium, calcium, magnesium, sulphur, zinc, manganese, copper, iron and sodium) which, after anaerobic biodigestion, can still be used for fertirrigation, since the digestion processes does not substantially decrease the mineral content. Attempts have been made to aggregate economic value to the liquid residue by considering its utilization as a fertilizer, herbicide, insecticide (Pothiraj *et al.*, 2006), nematicide, biosurfactant or substrate for microorganism growth (Ponte, 2001). The production of biogas, singlecell oil, microbial protein and recently, aromas can be sited as examples of its use (Barana, 2000).

2. VALUE-ADDING OF CASSAVA WASTE PRODUCTS

The dwindling food and feed reserves in the world have increased interest in the exploitation of carbohydrate residues that at present largely go to waste and are pollution hazards. The conversion of organic waste and residues into livestock feed reduces the environmental hazards associated with crop and agro-industrial wastes. Within the past decade fresh impetus has been given to the serious study of these carbohydrate residues as substrates for the production of protein enriched foods or feeds through microbial fermentation and other forms of value addition. Part of this impetus has stemmed from wider recognition of malnutrition in the developing countries and efforts to combat it. At the same time, with the ever-increasing seriousness of the waste problems from the processing of food and natural carbohydrate sources, the production of microbial protein from these wastes and by-products could be a profitable way of overcoming this difficulty. Carbohydrate residues are available in large quantities in many parts of South-East Asia. Some of these residues have been used as substrates to grow microorganisms, and their nutritive value has been documented (Okigbo and Anyaegbu, 2021). In some countries like Malaysia, and in many of her neighbouring countries and in Africa, there are increasing needs for protein sources. Protein consumption has been reported to be about 45 g/day/person and to

consist of not more than 17 g of animal protein (Anyaegbu *et al*., 2019). Efforts have been made to increase animal protein sources, such as meat from poultry and beef. Realizing these facts, considerable research has been conducted and is currently being intensified to maximize the use of various agro-industrial wastes, including those of carbohydrate residues, for useful animal feed and thus, indirectly, for food. Value addition of cassava and cassava wastes is necessitated by rapid post-harvest spoilage, deterioration, and environmental pollution caused by the effluent and the other associated wastes that poses aesthetic nuisance. Similarly, biogas plants of all sizes and varying levels of technical sophistication not only recover the energy contained in cassava wastes but also eliminate most of the animal and human health problems associated with it.

3. MATERIALS AND METHODS

Study Site

This was carried at Nnamdi Azikiwe University, Awka permanent site in the Microbiology laboratory.

Sample collection

Cassava wastes (peels) from three (3) different varieties of cassava (TMS/98/0581, TME/98/419 and TMS/98/30572) were obtained from local farmers (processors) at Mgbakwu, Awka North, Anambra State, Nigeria. The cassava varieties were identified/authenticated by the farm manager at the National Rooot Crop Research Institute (NRCRI), Igbariam, Anambra State, Nigeria. After the identification, they were transported to the Microbiology Laboratory Nnamdi Azikiwe University, for study.

Production of glucose syrup

A suspension of 35% (w/v) of previously extracted cassava starch was prepared. The suspension pH was adjusted using 0.2 M pH 6.0 phosphate buffer. This suspension was maintained under heating, increasing the temperature in 1 °C/minute, until the starch was completely solubilized. After, it was cooled down, the solution was incubated in water bath at 50 °C with 10 mL of commercial α-amylase Sigma (1 mg.mL–1) for 2 hours for total liquefaction. For scarification, this resulting maltodextrin syrup had its pH adjusted to 4.2 with 0.2 M of acetate buffer. Then, 100 mL of previously produced and characterized glucoamylase solution was added and incubated at 60 °C for 48 hours under constant agitation. The glucose production was monitored using the glucose oxidase method as before. After this process, the resulting syrup was purified by ion exchanged chromatography using Amberlite Ira 120 (Cationic resin) and Amberlite Ira 410 (Anionic resin) and then concentrated by evaporation under reduced pressure.

Isomerization of glucose syrup

The glucose syrup was filtered and concentrated to 40% solids in an oven at 60^{o}C. The pH of the concentrated syrup was adjusted to 7.5 and kept in the water bath at 60^{y}C. After equilibration, sweetzme (an enzyme) was added and incubated at 60^{o}C for 45 hour in the water bath. The fructose that was formed was estimated by the cysteine carbozole method. An aliquot (1.0ml) of the isomerized syrup was diluted 400 times and treated with 0.2 of cysteine hydrochloride (1.5%); 6.0 ml H_2SO_4 (70%) and 0.2 ml absolute

alcohol (0.12%) (w/v). After mixing for 10 seconds carbazole reagent was added and incubated for 1h at 37^{o}C. The absorbance of the coloured product was measured against a reagent blank at 500nm and the fructose yield was calculated using D-fructose standard.

Production of fructose syrup

Production of fructose syrup was carried out using previously obtained glucose syrup. The solution had its pH adjusted to pH 8.0 with 0.2 M Tris-HCl buffer containing MgSO4 .7H2 O 50 mM and CoCl2 .6H2 O 0.25 mM. Differently glucose syrup isomerization was performed using Streptomyces mycelium instead of the enzyme solution. For that, 30 g of Streptomyces sp. mycelium was fastened to dialysis bags to avoid contaminating the solution. The system was incubated under constant agitation at 70 °C for 96 hours. The fructose production was monitored using the cysteinecarbazol method proposed by Dishe and Borenfreud (1951).

Statistical Analysis

Data generated were represented as Mean ± Standard Deviation. The results were analyzed statistically using Analysis of Variance (ANOVA) and Paired t – test. P-value of < 0.05 was considered statistically significant.

4. RESULTS

Table 1: Reducing Sugar Content of Cassava waste (Peels) during Solid State fermentation for Treated Sample.

Organism	Fermentation Period (Days)					
	0	3	6	9	12	15
TMS/98/0581						
Aspergillus niger		18	40	36	34	32
Aspergillus flavus	2.5	22	42	40	38	35
Mixed culture		25	60	55	52	50
TME/98/419						
Aspergillus niger		16	29	27	24	19
Aspergillus flavus	1.53	18	40	36	33	30
Mixed culture		23	54	46	36	33
TMS/98/30572						
Aspergillus niger		14	26	19	15	12
Aspergillus flavus	1.37	16	38	32	27	25
Mixed culture		22	50	42	30	27

- Results are mean ± standard error of mean of the three replicates.

TMS - Tropical *manihot* species, a prefix for all cassava lines developed by IITA

TME – Tropical *manihot esculenta*, a prefix for all IITA land race accessions

Table 1 shows the production of reducing sugar from starch during solid state fermentation of treated cassava waste (peels). It was observed that all the isolates have the capacity to convert starch to reducing sugar at their different rate. The maximum value of reducing sugar was obtained at the 6^{th} day (60) from the mixed culture in variety 1 which is the peak of fermentation. Also, the table showed that the mixed culture yields greater reducing sugar followed by *Aspergillus flavus* and the least is *Aspergillus niger*.

Furthermore, in the bioconversion of starch to reducing sugar, it was also observed that the interaction between the isolates and cassava variety TMS/98/0581 yield higher reducing sugar followed by the interaction between isolates and cassava variety TME/98/419 and the least is the interaction between isolates and cassava variety TMS/98/30572.

Table 2: Reducing Sugar Content of Cassava waste (Peels) during Solid State fermentation for Untreated Samples

Organism	Fermentation Period (Days)					
	0	3	6	9	12	15
TMS/98/0581						
Aspergillus niger		4	13	22	18	14
Aspergillus flavus	2.5	10	17	26	24	22
Mixed culture		13	22	32	30	24
TME/98/419						
Aspergillus niger		7	13	22	18	16
Aspergillus flavus	1.53	8	16	23	21	18
Mixed culture		12	18	28	24	22
TMS/98/30572						
Aspergillus niger		6	12	19	17	13
Aspergillus flavus	1.37	7	13	22	18	16
Mixed culture		10	18	27	25	23

- Results are mean ± standard error of mean of the three replicates.

TMS - Tropical *manihot* species, a prefix for all cassava lines developed by IITA

TME – Tropical *manihot esculenta*, a prefix for all IITA land race accessions

Table 2 highlights the production of reducing Sugar from starch during solid state fermentation of untreated cassava waste (peels). From the table, all the isolates have the capacity to convert starch to reducing sugar at their different rates.

Also from the table, in the bioconversion of starch to reducing sugar, the mixed culture had the best performance followed by single culture of *Aspergillus flavus* and the least is *Aspergillus niger*.

Furthermore, it was observed that the interaction between the isolates and TMS/98/0581 produced better yield than TME/98/419 and TMS/98/30572.

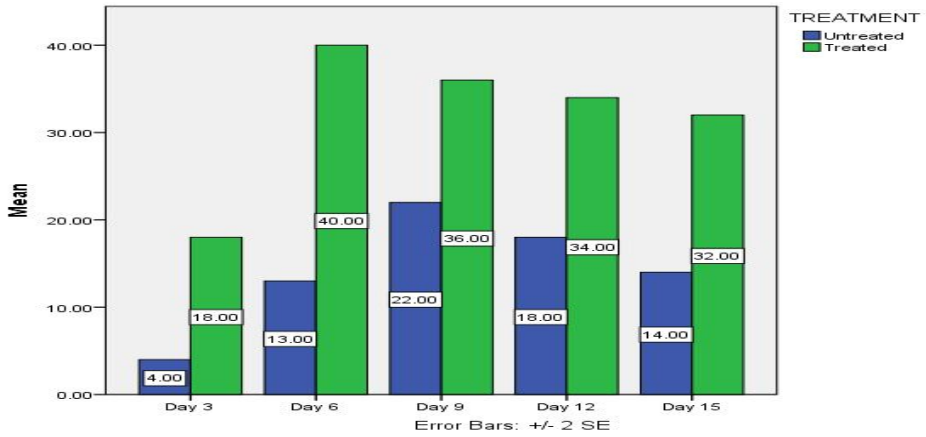

Figure 1: Reducing sugar content of TMS/98/0581 cassava waste (peels) during solid state fermentation of untreated and treated samples for *Aspergillus niger*(A graph of starch disappearance against days).

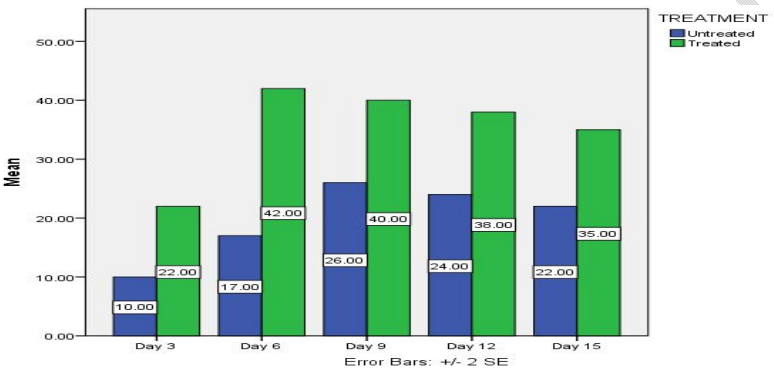

Figure 2: Reducing sugar content of TMS/98/0581 cassava waste (peels) during solid state fermentation of untreated and treated samples for *Aspergillus flavus*(A graph of starch disappearance against days).

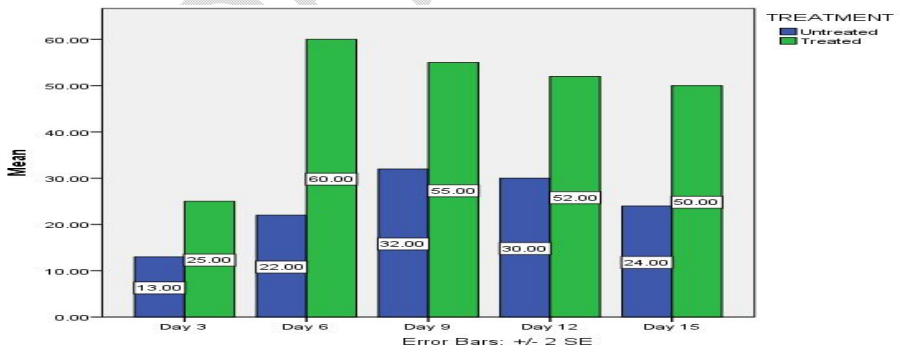

Figure 3: Reducing sugar content of TMS/98/0581 cassava waste (peels) during solid state fermentation of untreated and treated samples for mixed culture(A graph of starch disappearance against days).

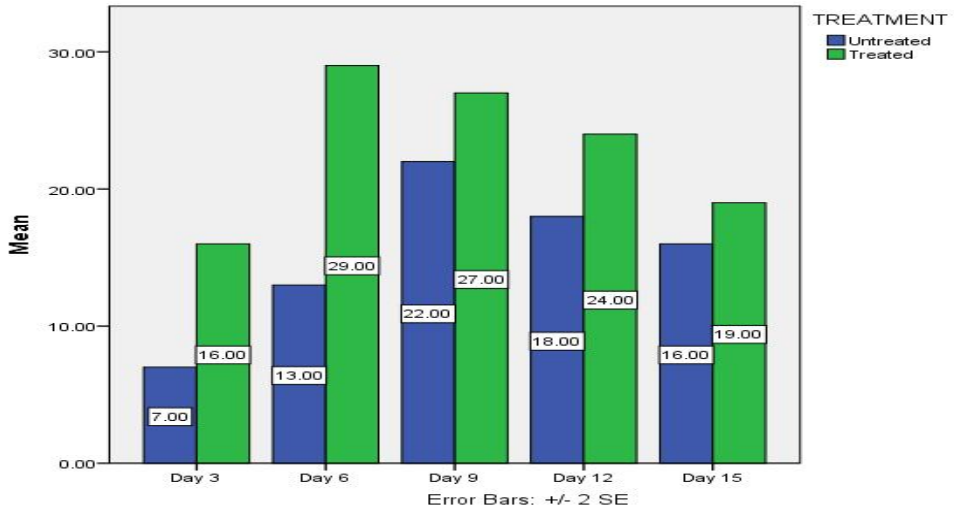

Figure 4: Reducing sugar content of TME/98/419 cassava waste (peels) during solid state fermentation of untreated and treated samples for *Aspergillus niger* (A graph of starch disappearance against days).

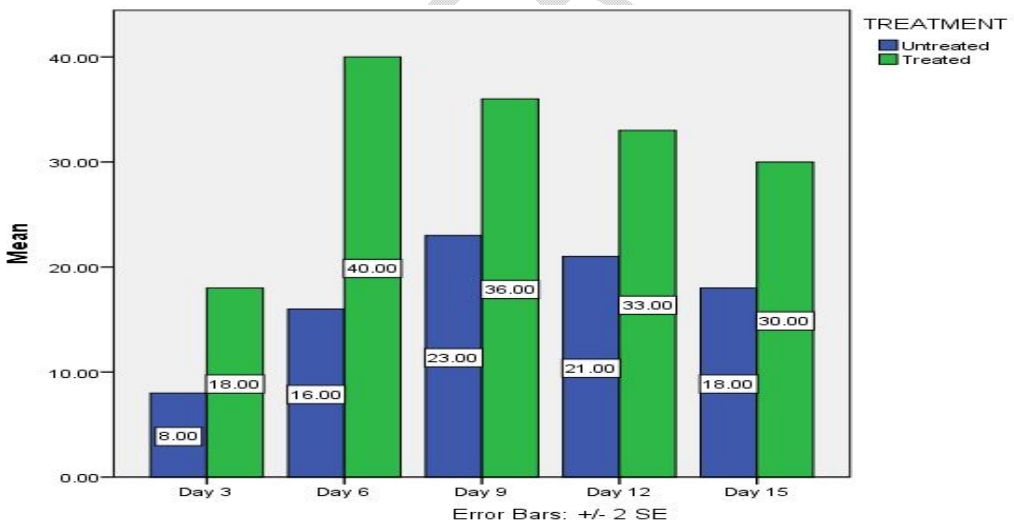

Figure 5: Reducing sugar content of TME/98/419 cassava waste (peels) during solid state fermentation of untreated and treated samples for *Aspergillus flavus*(A graph of starch disappearance against days).

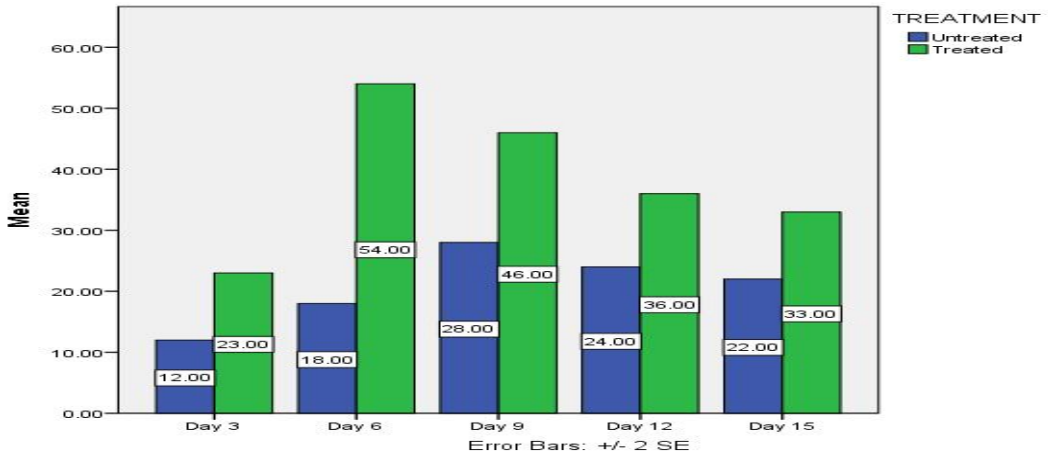

Figure 6: Reducing sugar content of TME/98/419 cassava waste (peels) during solid state fermentation of untreated and treated samples for mixed culture(A graph of starch disappearance against days).

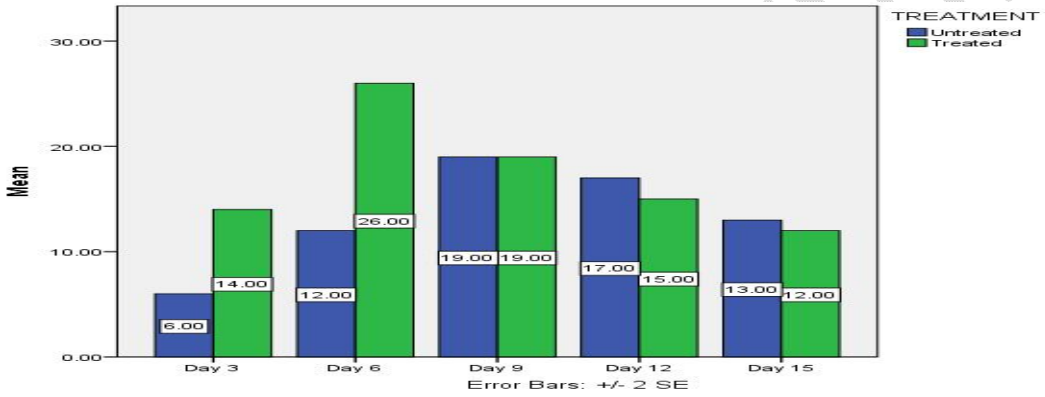

Figure 7: Reducing sugar content of TMS/98/30572 cassava waste (peels) during solid state fermentation of untreated and treated samples for *Aspergillus niger*(A graph of starch disappearance against days).

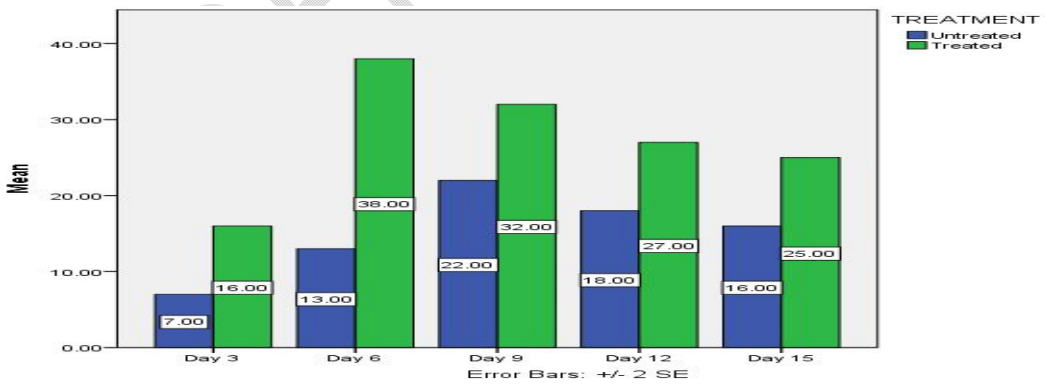

Figure 8: Reducing sugar content of TMS/98/30572 cassava waste (peels) during solid state fermentation of untreated and treated samples for *Aspergillus flavus*(A graph of starch disappearance against days).

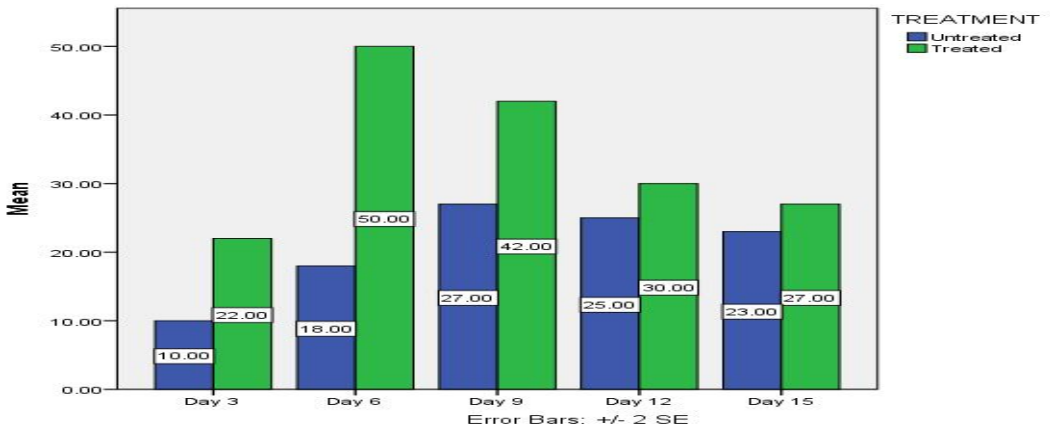

Figure 9: Reducing sugar content of TMS/98/30572 cassava waste (peels) during solid state fermentation of untreated and treated samples for mixed culture(A graph of starch disappearance against days).

5. DISCUSSION

The production of reducing sugar from starch during solid state fermentation of untreated and treated cassava waste revealed that 3rd day was the peak of fermentation as shown in figure 1. The single culture of *Aspergillus flavus* was observed to yields higher percentage of reducing sugar than the mixed culture. The cassava variety TME/98/419 showed better performance in the bioconversion of starch to reducing sugar at the 3rd day of fermentation having 35.8 and 24.0 for both untreated and treated cassava respectively. Statistics revealed that the figure tested significantly different between the values obtained by the isolates and the varieties of cassava at $P < 0.05$.

The production of reducing sugar from starch during solid state fermentation of untreated and treated cassava waste showed that the peak of fermentation was on the 3rd day as shown in figure 2. Statistics revealed that the figure tested significantly different between the values obtained by the isolates and the varieties of cassava at $P < 0.05$. The mixed culture was observed to yields lowest percentage of reducing sugar (33.9 and 23.8) for untreated and treated cassava respectively having single culture of *Aspergillus niger* as the highest (39.5 and 29.2) for both the untreated and treated cassava respectively.

The production of reducing sugar from starch during solid state fermentation of untreated and treated cassava waste (peels) as revealed in figure 3, showed that 3rd day was the peak of fermentation. The results were observed to yield similar results with other cassava varieties. The single culture of *Aspergillus niger* yield higher percentage of reducing sugar than mixed culture. The interaction between the *Aspergillus niger* and cassava variety TMS/98/30572 performed better than *Aspergillus niger* and cassava variety TME/98/419 as seen in percentage saccharification 72.0% and 71.5% for untreated and 75.1% and 74.6 for treated respectively.
However, statistical analysis using paired t – test showed significant difference between the mean value obtained from untreated and treated cassava waste in bioconversion of starch to reducing sugar at $P < 0.05$. From the figure 3 in the result, the initial increase and further decrease of reducing sugar in the fermentation might be due to simultaneous hydrolysis of starch to simple sugar and subsequent utilization

of the sugar by the isolates (Olanibiwoninu and Odunfa, 2012).). The mixed culture in both untreated and treated substrate was observed to produce higher yield of reducing sugar, similar results have been reported earlier with studies on cassava waste (Onilude, 2006) who attributes that higher yield of reducing sugar is correlated with higher cassava utilizing potentials of the isolates.

The production of reducing sugar from starch during solid state fermentation of untreated and treated cassava waste (peels) showed that 3rd day was the peak of fermentation as shown in figure 4. The results were observed to yield similar results with other cassava varieties. The single culture of *Aspergillus flavus* yield higher percentage of reducing sugar than *Aspergillus niger*. The interaction between the *Aspergillus flavus* and cassava variety TMS/98/30572 gave similar results as *Aspergillus flavus* and cassava variety TME/98/419 as seen in percentage saccharification 74.0% and 74.1% for untreated and 76.1% and 76.3 for treated respectively.
However, statistical analysis using paired t – test showed significant difference between the mean value obtained from untreated and treated cassava waste in bioconversion of starch to reducing sugar at $P < 0.05$. The initial increase and further decrease of reducing sugar in the fermentation as revealed in figure 4 in the result might be due to simultaneous hydrolysis of starch to simple sugar and subsequent utilization of the sugar by the isolates (Ezeogo *et al*., 2021). The mixed culture in both untreated and treated substrate was observed to produce higher yield of reducing sugar, similar results have been reported earlier with studies on cassava waste, who attributes that higher yield of reducing sugar is correlated with higher cassava utilizing potentials of the isolates (Nwakoby *et al*., 2021).

The production of reducing sugar from starch during solid state fermentation of untreated and treated cassava waste (peels) revealed that 3rd day was the peak of fermentation as shown in figure 9. The results were observed to yield similar results with other cassava varieties. The single culture of *Aspergillus flavus* yield higher percentage of reducing sugar than *Aspergillus niger*. The interaction between the *Aspergillus flavus* and cassava variety TMS/98/30572 gave similar results as *Aspergillus flavus* and cassava variety TME/98/419 as seen in percentage saccharification 74.0% and 74.1% for untreated and 76.1% and 76.3 for treated respectively.
However, statistical analysis using paired t – test showed significant difference between the mean value obtained from untreated and treated cassava waste in bioconversion of starch to reducing sugar at $P < 0.05$. From the above figures, the initial increase and further decrease of reducing sugar in the fermentation might be due to simultaneous hydrolysis of starch to simple sugar and subsequent utilization of the sugar by the isolates (Nwakoby *et al.*, 2021). The mixed culture in both untreated and treated substrate was observed to produce higher yield of reducing sugar, similar results have been reported earlier with studies on cassava waste (Nwakoby *et al.,* 2021) who attributes that higher yield of reducing sugar is correlated with higher cassava utilizing potentials of the isolates.

The production of reducing sugar from starch during solid state fermentation of untreated cassava waste (peels) as shown in table 3 was observed to yield similar results with the treated sample but at a lower yield. The mixed culture yield higher percentage of reducing sugar than the single culture. The interaction between the mixed culture and TMS/98/0581 performed better than TME/98/419 and TMS/98/30572, which releases 32%, 28% and 27% reducing sugar respectively. However, statistical analysis using paired t-test shows significant difference between the mean value obtained from treated and untreated cassava waste in bioconversion of starch to reducing sugar at $P < 0.05$. From the above table, the initial increase

and further decrease of reducing sugar in the fermentation might be due to simultaneous hydrolysis of starch to simple sugar and subsequent utilization of the sugar by the isolates. (Ezeogo *et al*., 2021). The mixed culture in both treated and untreated substrate was observed to produce higher yield of reducing sugar. Similar results have been reported earlier with studies on cassava waste. (Anyaegbu *et al*., 2019) who attributes that higher yield of reducing sugar is correlated with higher cassava utilizing potentials of the isolates.

The production of reducing sugar from starch during solid state fermentation of treated cassava waste revealed that 6^{th} day was the peak of fermentation as shown in Table 1.
Also, statistics revealed that the figure tested significantly different between the values obtained by the isolates and the varieties of cassava at $P < 0.05$. The mixed culture was observed to yield higher percentage of reducing sugar, followed by single culture of *Aspergillus flavus* and then *Aspergillus niger*. The TMS/98/0581 showed better performance than TME/98/419 and TMS/98/30572 in bioconversion of starch to reducing sugar. This was observed in the interaction between mixed culture and TMS/98/0581 which releases 60%, 54% and 50% reducing sugar for TME/98/419 and TMS/98/30572 respectively at the 6^{th} day fermentation.

CONCLUSION

Natural fermentation of plant materials is widely used to transform and preserve plant products and improve the organoleptic properties of the final products. Fermentation is one of the oldest and most important traditional food processing and preservation techniques. Food fermentations involve the use of microorganisms and enzymes for the production of foods with distinct quality attributes that are quite different from the original agricultural raw material. Food fermentations involve the action of microorganisms, and consequently, the environmental conditions should be controlled to ensure the growth of favourable microbial species for the development of desired qualities of the finished food products. Fermentation enhances the nutrient content of foods through the biosynthesis of vitamins, essential amino acids and proteins. It also enhances micronutrient bioavailability and aids in degrading anti-nutritional factors. Fermentation is a common traditional household technology in many parts of the world. Regional differences occur in manufacturing practices, consumption habits, quality and level of acceptability of fermented foods. Many African foods are fermented before consumption. Our local food is said to be fermented when it has undergone some chemical changes which make it acceptable and ready for consumption.

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