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Analysis of Starch Content of Cassava Waste (Peels) during Solid State Fermentation of Untreated and Treated Sample

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Casava (*Manihot esculenta*) is one of the most important crop source of carbohydrate and its production is vital to the economy of Nigeria as a country. Nigeria is the world's largest producer of the commodity. This research work was done to determine the utilization of casava waste (peel) by fungi to produce reducing sugar (fructose).The bioconversion potentials and growth of fungi on varieties of casava waste (peels) during solid state fermentation were assessed. The myco-utilization of casava wastes were carried out by evaluating treated casava waste and untreated casava waste in solid state fermentation with mixed culture of *Aspergillus niger* and *Aspergillus flavus,* single culture of *Aspergillus niger* and *Aspergillus flavus.* The results revealed that the interaction between the mixed culture and *Aspergillus niger* and *Aspergillus flavus* and cassava variety TMS/98/0581 of treated cassava waste yielded better performance in protein enhancement

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and reducing sugar production than the single culture and other cassava varieties. Starch content (%) of Cassava waste during solid state fermentation of Treated Sample showed that the mixed culture of *Aspergillus niger* and *Aspergillus flavus* has the highest starch saccharification, 87.0%, 80.7% and 81.0% for casava waste TMS/98/0581, TME/98/419 and TME/98/30572 respectively having single culture of *Aspergillus niger* as the lowest starch saccharification, 80.3%, 74.6% and 75.1% for cassava waste TMS/98/0581, TME/98/419 and TMS/98/30572 respectively. Also, starch content (%) of mixed cassava waste (peels) during Solid State fermentation of Untreated Sample showed that the mixed culture of *Aspergillus niger* and *Aspergillus flavus* has the highest starch saccharification, 77.5%, 76.3% and 76.1% for casava waste TMS/98/0581, TME/98/419 and TMS/98/30572 respectively having single culture of *Aspergillusniger*, 71.5% and 72.0% for cassava waste TME/98/419 and TMS/98/30572 respectively and single culture of *Aspergillus flavus*, 74.0% for cassava waste TMS/98/0581 as the lowest starch saccharification.

Keywords: Cassava peel; fermentation; fungi; isolation.

1. INTRODUCTION

Cassava (*Manihot esculenta*) production is vital to the economy of Nigeria as a country. Nigeria is the world's largest producer of the commodity. The crop is produced in 24 of the country's 36 states. In 1999, Nigeria produced 33 million tonnes, while a decade later, it produced approximately 45 million tonnes, which is almost 19% of production in the world [1]. The average yield per hectare is 10.6 tonnes [1]. 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 [2]. Cassava is processed in many processing centres and fabricating enterprises set up in the country.

Originally a crop of South America, it was introduced into Nigeria's southern part during the period of slave trade proliferated by Portuguese explorers and colonizers in the sixteenth century [1]. However, its importance to the country got a boost in the late nineteenth century when more formerly enslaved Nigerians returned to their homeland and introduced processing techniques. Over the years, it has become a major economic sustenance crop and it has attained the status of largest produce in Nigeria with recorded production of 34 million tones per a year and is a cash crop of great importance to the people of Nigeria [3,4]. Though the crop is produced in 24 of the country's 36 states, cassava production dominates the southern part of the country, both in terms of area covered and number of farmers growing the crop. Planting occurs during four planting seasons in the various geo-ecological zones. The major states of Nigeria which produce cassava are Anambra, Delta, Edo, Benue, Cross River, Imo, Oyo, and Rivers, and to a lesser extent Kwara and Ondo.

In 1999, Nigeria produced 33 million tonnes. As of 2000, the average yield per hectare was 10.6 tonnes. Cassava is grown throughout the year, making it preferable to the seasonal crops of yam, beans or peas. It displays an exceptional ability to adapt to climate change, with a tolerance to low soil fertility, resistance to drought conditions, pests and diseases, and suitability to store its roots for long periods underground even after they mature [5]. Use of fertilizers is limited, and it is also grown on fallow lands. Harvesting of the roots after planting varies from 6 months to 3 years [5]. The land holding for farming in Nigeria is between 0.5–2.5 hectares (1.2–6.2 acres), with about 90% of producers being small-scale farms [1]. In order to increase production, several varieties of cassava have been developed which are pest resistant; production in the country is hampered with problems with green mite, the cassava mealybug, and the variegated grasshopper. Diseases affecting cassava crop are mosaic disease, bacterial blight, anthracnose, and root rot [1,5].

Eager to promote self-sufficiency, the government wants to promote the use of cassava while curtailing rice and wheat imports. According to a Nigerian Presidential Initiative of July 2002, the cropped area of cultivation of cassava was proposed to be increased to 5 million hectares by the end of 2010 with a projected annual yield of 150 million tonnes resulting in an annual export earning of US$5 billion. An adopted innovation is the introduction of vitamin A-rich cassava. The Federal Government of Nigeria launched a project to introduce pro-Vitamin A cassava varieties to 1.8 million farmers in the country [6].

Cassava, which is rich in starch in the form of carbohydrate, has multiple uses. It is consumed in many processed forms, in the industry and also as livestock feed [1]. Roots are made into flours. Flours are of three types, yellow garri, white garri, or intermediate colour, with yellow garri considered the best product in Nigeria. Its other products are as dry extraction of starch, glue or adhesives, modified starch in pharmaceutical as dextrines, as processing inputs, as industrial starch for drilling, and processed foods [7].

In case of cellulosic wastes, cellulose is mainly converted to oligosaccharides, then to glucose by hydrolysis. Glucose is further converted to form other products including aldehydes and ketones, from which organic acids are produced. The hydrolysis of cellobiose was reported to be a second-order reaction. It was suggested that hydrolysis of cellobiose mainly took place by the nucleophilic attack of the oxygen atom of the water molecule or by the attack of a proton ion dissociated from supercritical water to the glycosidic carbon atom of the cellobiose molecule under the condition where the density of water was low and that the increase in the local water density around a solute promotes the hydrolysis rate at identical conditions.

The aim of this study is to determine the utilization of cassava waste (peel) by fungi to produce reducing sugar (fructose) while the objectives are to isolate and identify fungi associated with cassava wastes (peels) degradation, investigate the degradability of different varieties of cassava wastes (peels) by the fungal isolates.

2. MATERIALS AND METHODS

2.1 Procurement of Study Sample

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.

2.2 Substrate Preparation

The cassava roots peels were collected, washed thoroughly in tap water, air dried for seven days, then oven-dry at temperature of 50^{o}C for 48 hr and milled using a sterile electric blender (Sony), and kept in an air tight container until when needed.

2.3 Media Preparation

All culture media (0.1% peptone, Malt Extract Agar (MEA), Potatoes Dextrose Agar (PDA) and 60ml of minimal basal medium (1g/L $CaCl_2$. $7H_2O$, 1h/L $MgSO_{-4}$, 2g/L $(NH_4)_2$ SO_{-4} and 0.5g/L KH_2SO_4))that were used in this study were prepared according to the manufacturers' instructions.

2.4 Isolation

Fungi isolation was done using the method of Obadina et al., [8]. Five grammes (5g) of each of the solid cassava waste was aseptically homogenized in 50 ml of sterile 0.1% peptone water in conical flasks. A ten fold serial dilution was done in test tubes and 0.1ml of each of the samples was inoculated on duplicate plates of malt extract Agar (MEA) using the spread plate method. The inoculated plates were incubated at room temperature (28 ± 2^{o}C) for 4-5 days. The colonies that developed were purified by sub-culturing on Potato Dextrose Agar (PDA) and pure cultures were stored on PDA slants for identification.

2.5 Identification of Fungal Isolates

Slide culture of the fungal isolates was done and lactophenol cotton blue mount of the isolates were employed to observe the microscopic features of the isolates using the x40 objective lens. Identification of the isolates was based on the colony morphology, colony colour, age of culture and hyphal characteristics using the descriptions of fungal atlas [9].

2.6 Determination of Starch Content

2.5 g of sample was mixed with 50ml of water in 250ml of conical flask and allowed to stand for 1hr. 20ml of conc. HCL and 150ml of distilled water were also added to the conical flask, and then placed in water bath for 2 hrs. then allow the set-up to cool and neutralize with NAOH. The glucose content were determined using anthrone

reagent. Prepare series of glucose solution such that 1ml contains 0 –80mg; use these to calibrate the glucose standard curve. To 1ml each of standard solution and a test sample in test tubes add 5ml of anthrone reagent and mix properly in 100ml beaker. Cover the tube and boil in water bath for 20 minutes for colour to develop. Cool the tubes and read their absorbance at 620nm against a blank containing only 1ml of water 5ml anthrone reagent. The concentration of the test sample is observed from the absorbance by interpolation involving the concentration and dilutions made.

The mass of starch = Mass of glucose x 0.9.

$$\% \text{ Starch content } = \frac{A - B}{A} \text{ x } \frac{100}{1}$$

A = Starch content before fermentation
B = Starch content after fermentation

2.7 Solid State Fermentation of Cassava Peels

Solid state fermentation of cassava wastes (peels) was done according to the method of Pothiraj et al. [10]. Twenty grams (20g) of each cassava wastes (peel) treated and untreated were each placed in 250ml Erlenmeyer flasks. Distilled water (60ml) was added to the untreated cassava waste while 60ml of minimal basal medium (1g/L $CaCl_2$. $7H_2O$, 1h/L $MgSO_{-4}$, 2g/L $(NH_4)_2$ SO_{-4} and 0.5g/L KH_2SO_4) was added to the pre-treated cassava peels. The flasks were plugged with cotton wool and autoclaved at 121^oC for 15 minutes. This was allowed to cool. Agar blocks (8mm plug) containing seven days old cultures of the isolates were aseptically put into the individual flasks containing the substrate. This was done in triplicate and was incubated at room temperature (28 ± 2^oC) for 15 days, Bio-converted cassava peel samples was drawn at intervals of three days and analyzed for starch, reducing sugar and mycelia protein [10].

2.8 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.

3. RESULTS

Table 2 shows the starch utilizing potentials of the isolates and its interaction with cassava waste in untreated cassava peel. The table revealed that all the isolates have the capacity to utilize cassava waste but the mixed culture shows faster starch utilization rate than the single culture of *Aspergillus niger* and *Aspergillus flavus* which indicates its higher potentials in starch degradation. This is observed in the interaction between mixed culture and the three replicates of cassava, which showed higher starch saccharification rate when compared to the interaction between the single cultures. Statistics revealed that there is no significant difference between the values obtained by the isolates and the three replicate of cassava at $P<0.05$

Table 3 represents the utilization of treated waste peel by the fungal isolates. The table showed that all the isolates have the capacity to utilize cassava waste but the mixed culture showed better performance than the single culture of *Aspergillus niger* and *Aspergillus flavus* which indicates its higher potentials in starch degradation. Statistics showed that there is significant difference between the values obtained by the isolates and the cassava peels at $P<0.05$.

Table 1. Mean percentage composition of cassava waste (peels) before fermentation

Parameter	TMS /98/0581	TME/98/419	TMS/98/30572
Starch	64.0 ± 1.0	59.0 ± 1.2	61.0 ± 1.0
Protein	2.57 ± 0.6	2.30 ± 0.5	2.63 ± 0.6
Reducing Sugar	2.50 ± 0.5	1.53 ± 0.5	2.67 ± 0.1

❖ *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. Starch content (%) of cassava waste (peels) during solid state fermentation of untreated sample

Cassava / Organisms	Fermentation Period (Days)						
	0	**3**	**6**	**9**	**12**	**15**	**% Saccarification**
TMS/98/0581							
Aspergillus niger	42.8±0.5	28.7±0.7	23.6±0.5	18.4±0.3	16.4±0.5	74.4	
Aspergillus flavus	64.0	40.0±0.6	27.6±0.1	22.3±0.4	17.8±0.1	16.8±0.2	74.0
Mixed culture	35.0±0.3	21.8±1.1	18.6±0.5	17.4±0.6	14.4±0.5	77.5	
TME/98/419							
Aspergillus niger	39.5±0.5	32.0±0.5	24.4±0.4	18.9±0.6	16.8±0.5	71.5	
Aspergillusflavus	59.0	35.8±1.2	28.4±0.1	18.6±0.2	17.1±0.1	15.3±1.2	74.1
Mixed culture	33.9±0.1	20.9±0.2	16.4±0.1	15.1±0.5	14.0±0.5	76.3	
TMS/98/30572							
Aspergillus niger	38.5±0.1	28.7±0.1	22.5±0.9	20.0±0.5	17.2±0.4	72.0	
Aspergillus flavus	61.0	39.2±0.5	29.7±1.2	22.6±0.6	18.3±0.2	15.9±0.5	74.0
Mixed culture	33.6±0.4	20.9±0.5	18.2±0.2	16.3±0.5	14.6±0.4	76.1	

Key: 3, 6, 9, 12 and 15 represent days (Day 3, Day 6, Day 9, Day 12 and Day 15)

❖ *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 3. Starch Content (%) of Cassava waste during Solid State fermentation of Treated Sample

Cassava / Organism	Fermentation Period (Days)						
	0	**3**	**6**	**9**	**12**	**15**	**% Saccarification**
TMS/98/0581							
Aspergillus niger	31.0±0.6	17.4±0.5	16.2±0.5	14.6±0.5	12.6±0.4	80.3	
Aspergillus flavus	64.0	28.6±1.2	15.5±0.5	13.2±0.5	11.6±1.5	10.6±0.4	83.4
Mixed culture	23.0±0.6	12.1±0.1	11.5±0.4	10.2±0.5	8.5±0.5	87.0	
TME/98/419							
Aspergillus niger	29.2±1.2	21.8±0.8	18.9±0.8	16.0±0.7	15.0±0.5	74.6	
Aspergillus flavus	59.0	24.0±0.9	19.4±0.4	17.6±0.4	16.5±0.5	14.0±0.5	76.3
Mixed culture	23.8±0.8	17.2±0.5	14.5±0.4	12.5±0.4	11.4±0.6	80.7	
TMS/98/30572							
Aspergillus niger	32.0±1.0	19.6±0.1	17.5±0.5	16.8±0.4	14.2±0.1	75.1	
Aspergillus flavus	61.0	30.0±0.1	19.1±0.5	17.4±1.1	16.9±0.5	14.6±0.5	76.1
Mixed culture	25.3±0.2	17.7±0.5	15.3±0.1	14.3±1.0	11.8±0.5	81.0	

❖ *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

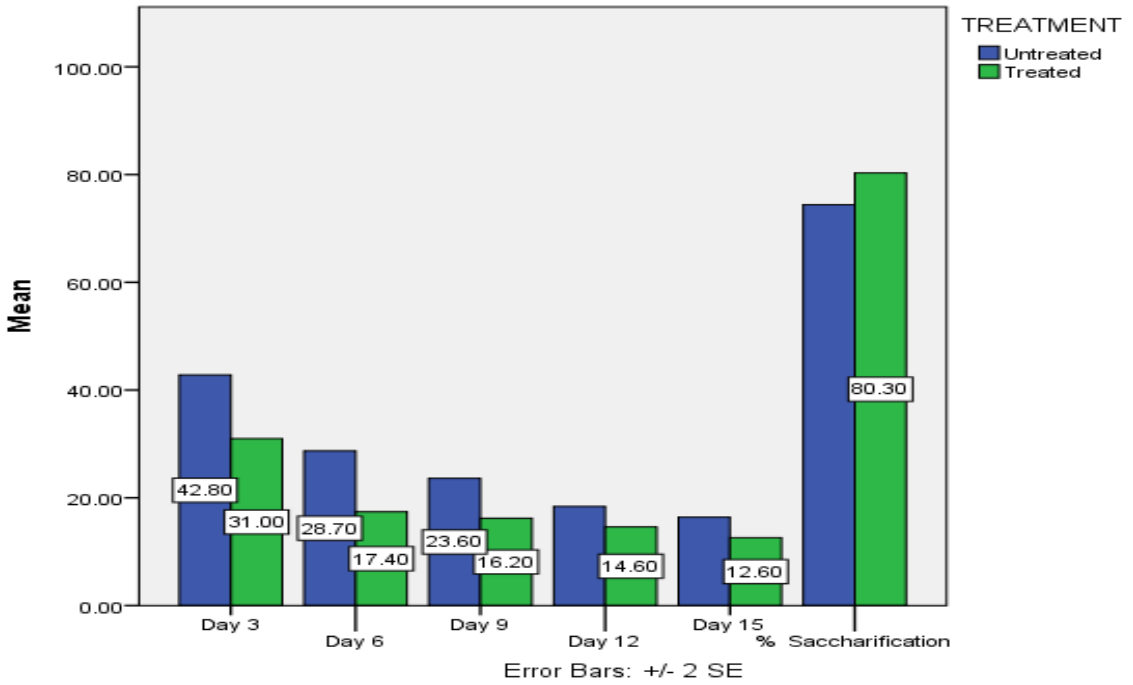

Fig. 1. A starch content (%) of TMS/98/0581 cassava waste (peels) during solid state fermentation of untreated and treated sample for *Aspergillus niger* (A graph of starch disappearance against days)

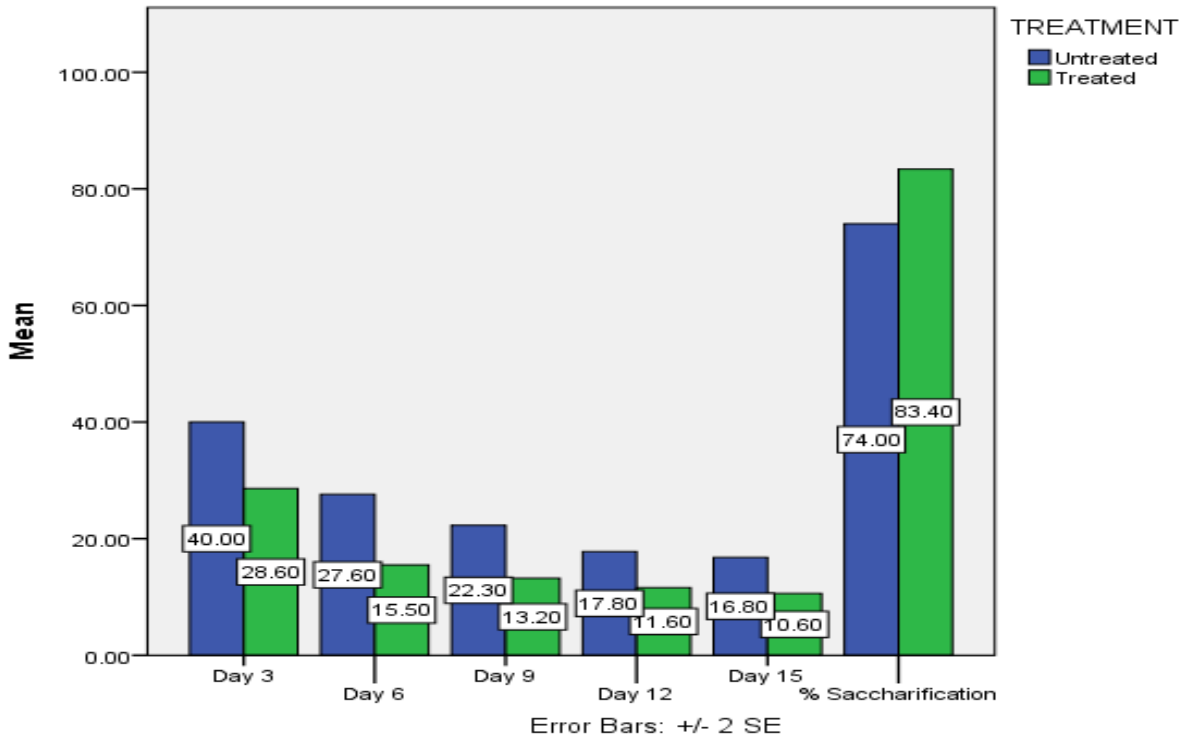

Fig. 2. A starch content (%) of TMS/98/0581 cassava waste (peels) during solid state fermentation of untreated and treated sample for *Aspergillus flavus* (A graph of starch disappearance against days)

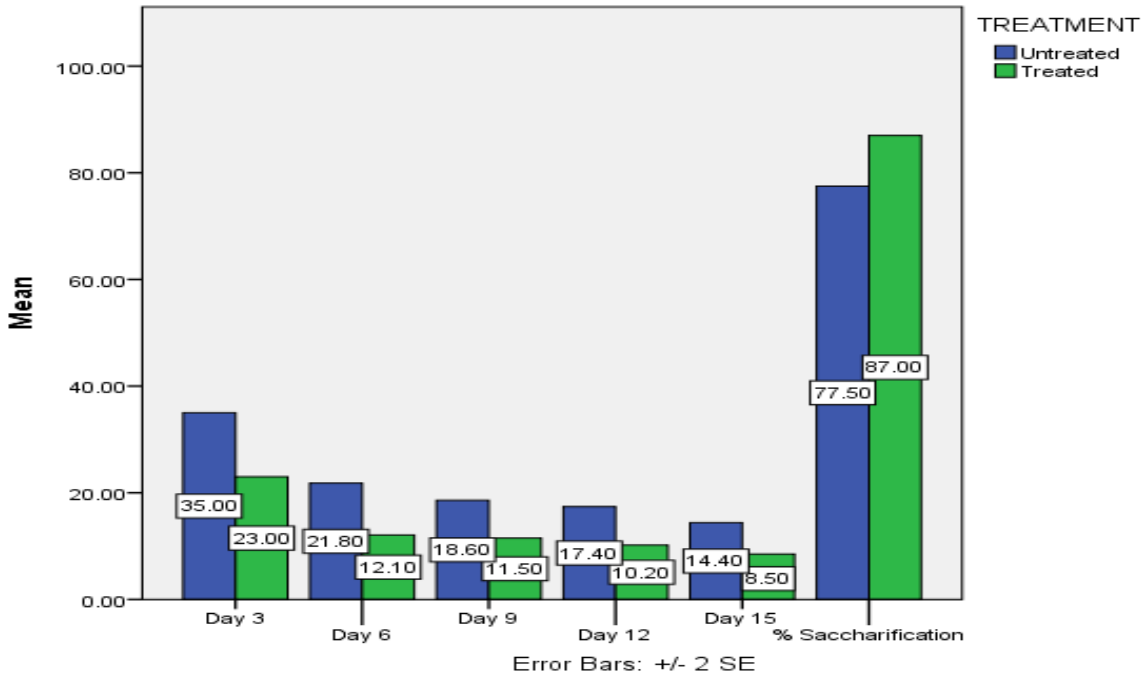

Fig. 3. A starch content (%) of TMS/98/0581 cassava waste (peels) during solid state fermentation of untreated and treated sample for mixed culture (A graph of starch disappearance against days)

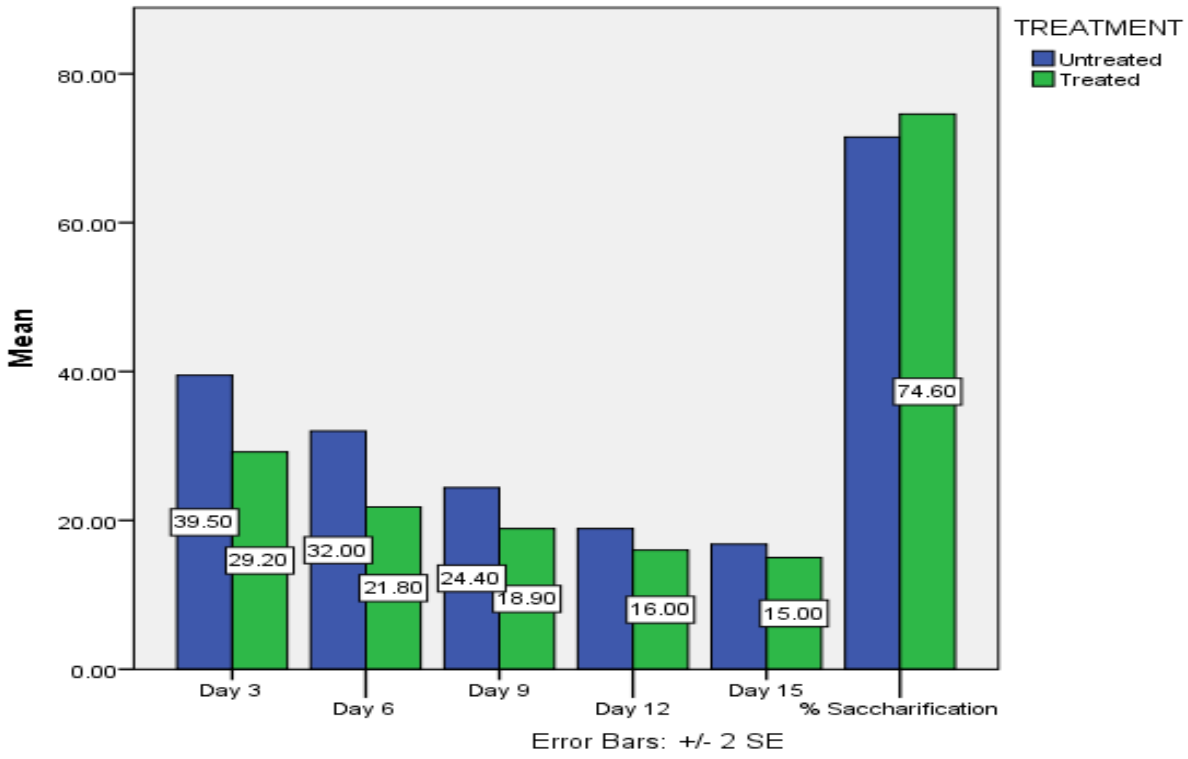

Fig. 4. A starch content (%) of TME/98/419 cassava waste (peels) during solid state fermentation of untreated and treated sample for *Aspergillus niger* (A graph of starch disappearance against days)

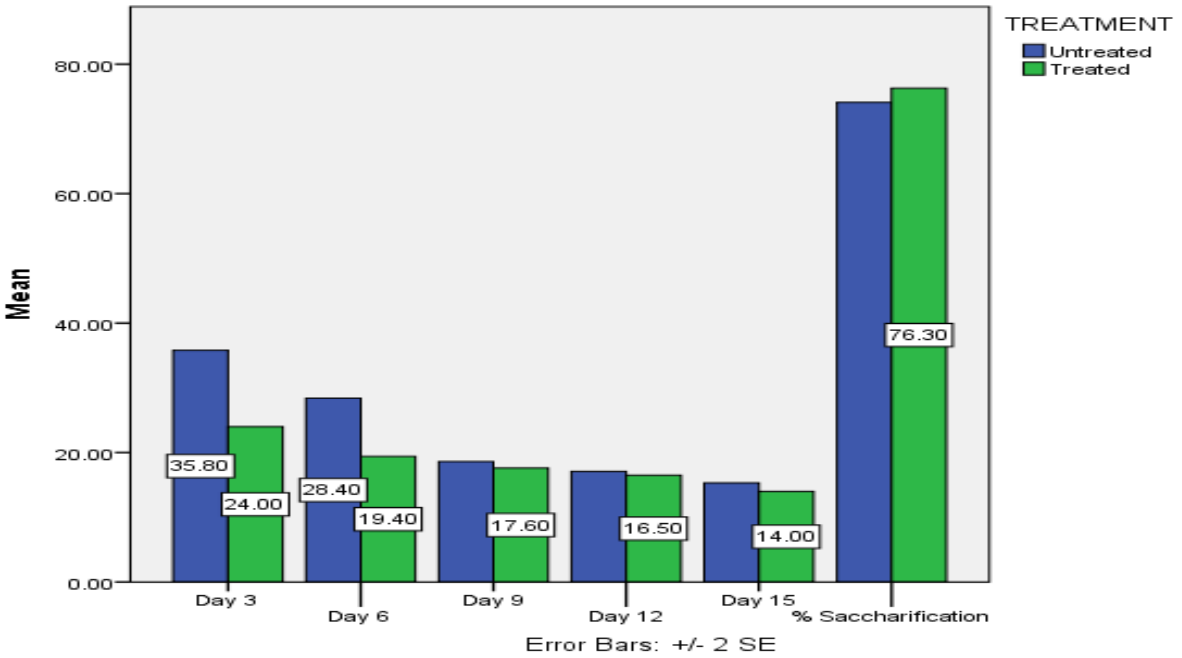

Fig. 5. A starch content (%) of TME/98/419 cassava waste (peels) during solid state fermentation of untreated and treated sample for *Aspergillus flavus* (A graph of starch disappearance against days)

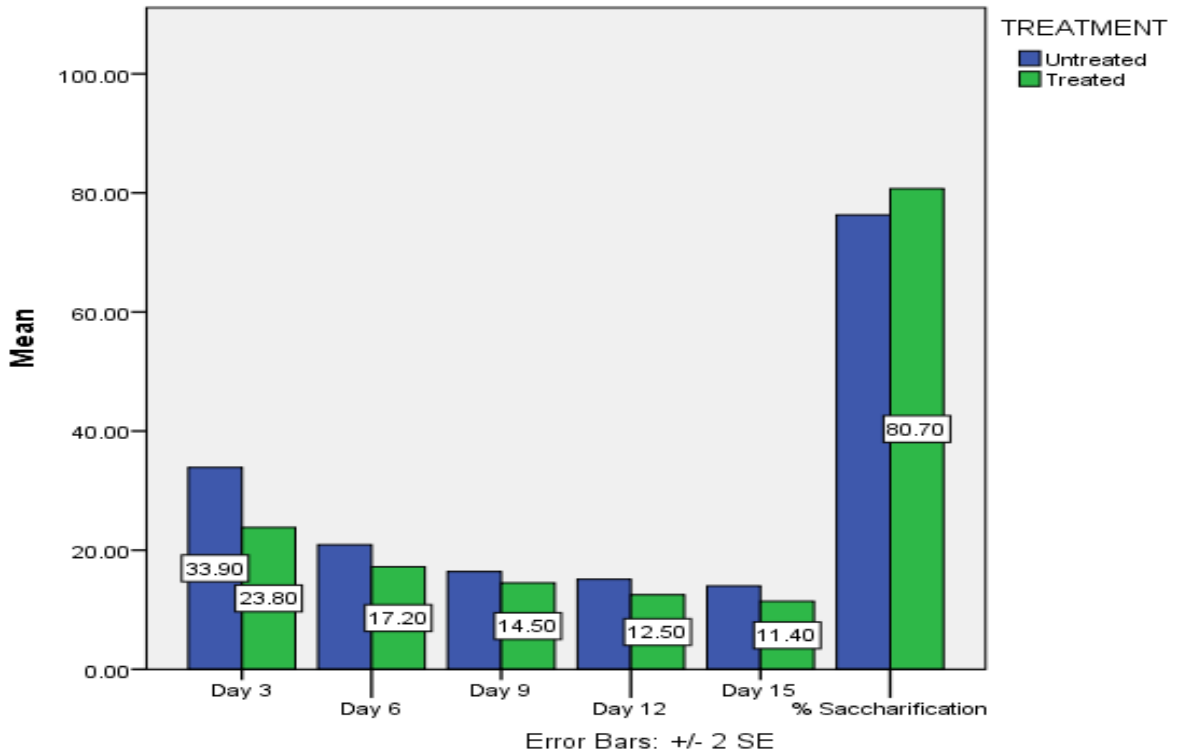

Fig. 6. A starch content (%) of TME/98/419 cassava waste (peels) during solid state fermentation of untreated and treated sample for mixed culture (A graph of starch disappearance against days)

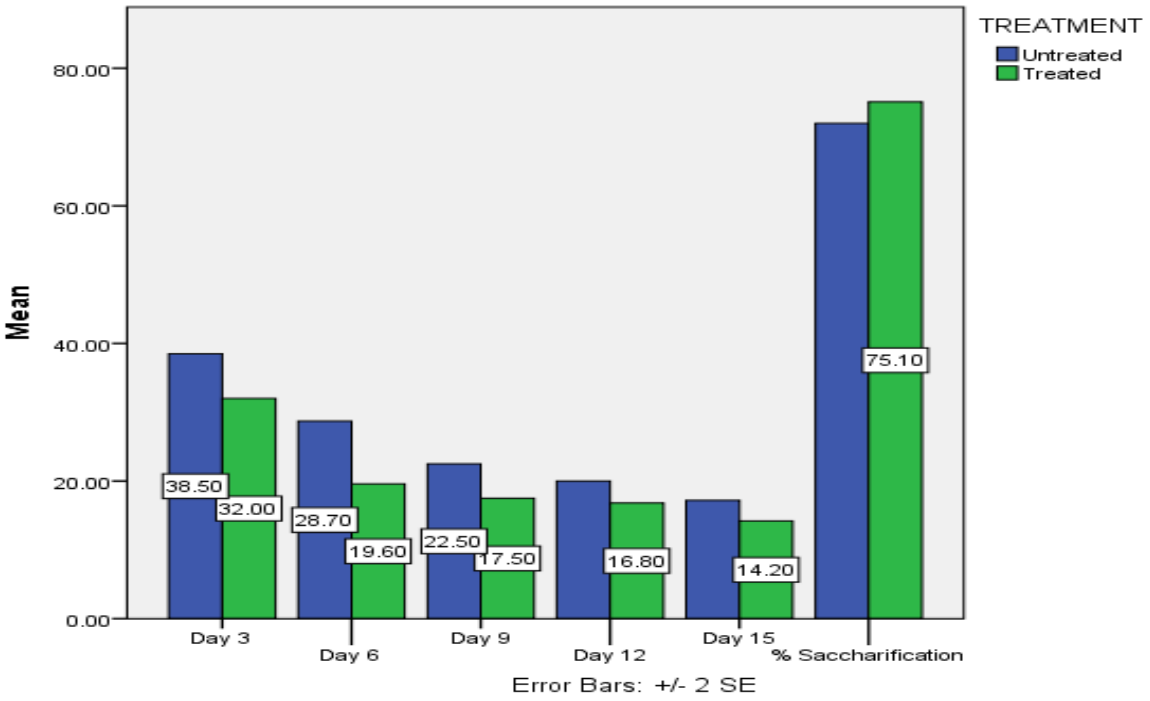

Fig. 7. A starch content (%) of TMS/98/30572 cassava waste (peels) during solid state fermentation of untreated and treated sample for *Aspergillus niger* (A graph of starch disappearance against days)

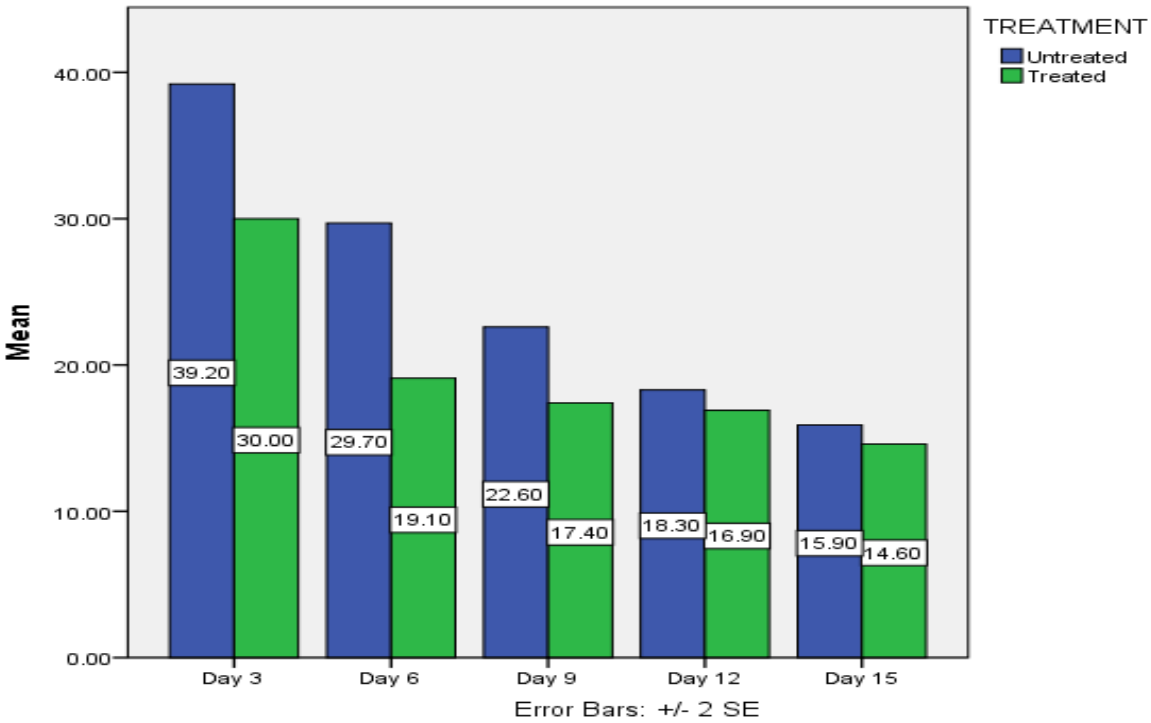

Fig. 8. A starch content (%) of TMS/98/30572 cassava waste (peels) during solid state fermentation of untreated and treated sample for *Aspergillus flavus* (A graph of starch disappearance against days)

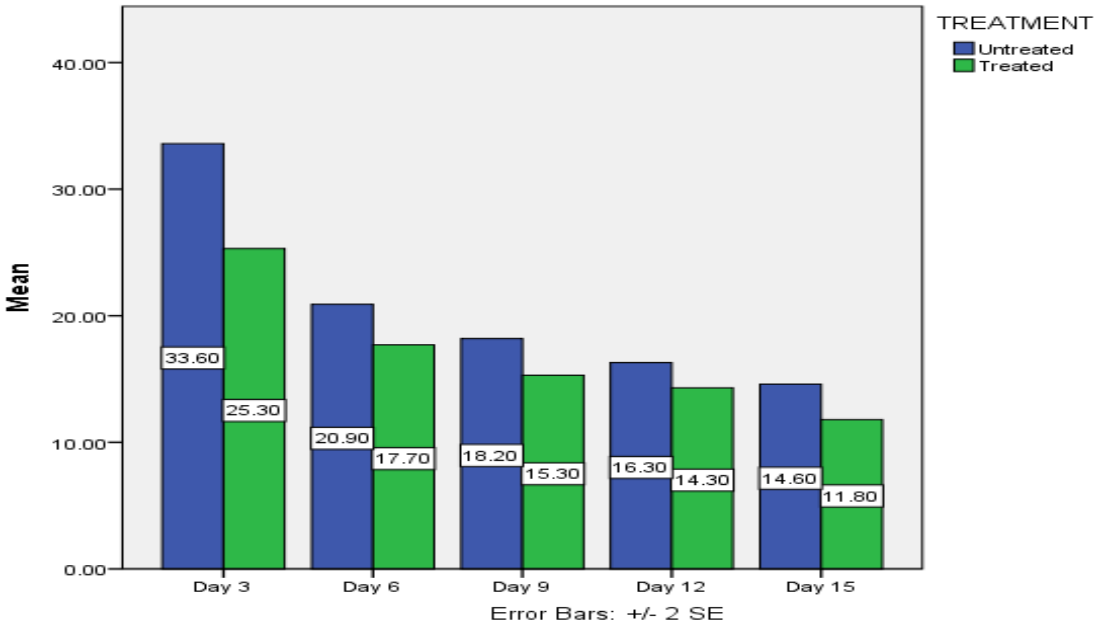

Fig. 9. A starch content (%) of TMS/98/30572 cassava waste (peels) during solid state fermentation of untreated and treated sample for mixed culture (A graph of starch disappearance against days)

4. DISCUSSION

A single culture of *Aspergillus niger*, *Aspergillus flavus* and a mixed culture of *Aspergillus niger* and *Aspergillus flavus* for the fermentation of three varieties (TMS/98/0581, TME/98/419 and TMS/98/30572) of cassava waste (peels) to evaluate the feasibility of the valued added products harnessed were used in this study. The mixed culture of *Aspergillus niger* and *Aspergillus flavus* has the highest and fastest utilization of starch than the single pure culture. However, statistics revealed that there is significant difference between the mean values obtained by the varieties of cassava at $P < 0.05$. Cassava TMS/98/0581 were utilized more by the isolates than TME/98/419 and TMS/98/30572, this was well observed in the interaction between the isolates and the TMS/98/0581 by saccharifying 87.0%, 83.4% and 80.3% of starch at the 15th day fermentation of the treated cassava waste by mixed culture, single culture of *Aspergillus flavus* and *Aspergillus niger* respectively as shown in Table 2. Furthermore, the mixed culture showed higher utilizing potential for starch degradation in all the varieties of cassava than the single culture.this result is in line with the works of Nwakoby et al. [11].

All the varieties of cassava were utilized by the isolates but the interaction between the *Aspergillus niger* of Cassava variety TMS/98/0581 yielded better performance having 42.8% and 31.0% for untreated and treated samples respectively at day 3 as the highest against 16.4% and 12.6% of untreated and treated samples respectively at day 15 as the lowest as shown in Fig. 1. Also, statistical analysis using paired t-test showed that there is significant difference between the values obtained by the treated cassava and the untreated cassava waste at $P < 0.05$ in starch utilization. Sequel to Tables 2 and 3 in the result, Starch utilization potential of these fungi can be correlated with the activities of their starch saccharification enzyme viz amylase activity. The successful degradation of cassava waste by single pure culture of *Aspergillus niger*, *Aspergillus flavus* and mixed culture could be attributed to their amylolytic potential. However, familiar result has been reported earlier with studies of *Aspergillus niger* grown on spent grain liquor [12].

The starch utilization of untreated and treated cassava waste (peels) showed that all the varieties of cassava were utilized by the isolates but the interaction between the *Aspergillus flavus* on Cassava variety TMS/98/0581 yielded better performance having 40.0% and 28.6% for untreated and treated samples respectively at day 3 as the highest against 16.8% and 10.6% of

untreated and treated samples respectively at day 15 as the lowest as shown in Fig. 2. Also, statistical analysis using paired t-test showed that there is significant difference between the values obtained by the treated cassava and the untreated cassava waste at $P < 0.05$ in starch utilization. Also, from Table 2 and Table 3 in the result, the activities of their starch saccharification enzyme viz amylase activity can be correlated with Starch utilization potential of these fungi [3,4]. The successful degradation of cassava waste by single pure culture of *Aspergillus niger*, *Aspergillus flavus* and mixed culture could be attributed to their amylolytic potential.

The starch utilization of untreated and treated cassava waste (peels) showed that all the varieties of cassava were utilized by the isolates but the interaction between the mixed culture TMS/98/0581 yielded better performance having 35.0% and 23.0% for untreated and treated samples respectively at day 3 as the highest against 14.4% and 8.5% of untreated and treated samples respectively at day 15 as the lowest as shown in Fig. 3. Also, statistical analysis using paired t-test showed that there is significant difference between the values obtained by the treated cassava and the untreated cassava waste at $P < 0.05$ in starch utilization.

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. 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 single culture of *Aspergillus niger* was observed to yields higher percentage of reducing sugar, followed by single culture of *Aspergillus flavus* and then 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 39.5 and 29.2 for both untreated and treated cassava respectively as shown in Fig. 8. This align with the work of Anyaegbu et al. [13,14].

5. CONCLUSION

The chemical composition of cassava waste (peels) can be greatly influenced by isolates as revealed in this study by releasing reducing sugar and increasing its protein content which shows the feasibility of bioconversion of cassava waste to valued added product. The actions of acid and enzyme hydrolysis have a remarkable capacity to degrade cassava peels for higher percentage production of reducing sugar and microbial protein than untreated cassava peels. Also, the mixed culture of *Aspergillus niger* and *Aspergillus flavus* was observed to have higher degree of protein and reducing sugar yield than pure single culture of *Aspergillus niger* and *Aspergillus flavus* during fermentation. However, the interaction of the mixed culture with the cassava variety TMS/98/0581 was also observed to yield higher values of microbial protein and reducing sugar than the other two varieties of cassava (cassava variety TME/98/419 and cassava variety TMS/98/3057) that was evaluated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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