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FUNGAL POPULATION AND PHYSICOCHEMICAL PROPERTIES OF BIOETHANOL PRODUCED FROM CASSAVA (*Manihot esculenta*)

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ABSTRACT

The quest for affordable and environmentally friendly fuel has led to the production of bioethanol from agricultural products. This study was undertaken to produce alcohol from cassava by the fermentative action of Aspergillus nigerand Saccharomyces cerevisiae. The fermentation process comprised of two (2) set ups- one was fermented by Aspergillus niger and the other was fermented by Saccharomyces cerevisiae. The fermentation process lasted for 12 days and it was distilled on the 14th day. The process was monitored and controlled by carrying out physicochemical (pH, temperature, specific gravity, alcohol content) and microbiological analyses using standard methods. There was a decrease in the pH for both set ups during the course of the fermentation. The temperature was between 25.0^{o}C to 30.5^{o}C for both setups. The specific gravity decreased during the course of the fermentation from 1.03 to 0.01 which led to an increase in the alcohol content from 0 to 55% for the setup that involved Aspergillus niger and 1.02 to 0.03 which led to an increase in the alcohol content from 0 to 50% for the setup that involved Saccharomyces cerevisiae. There was an increase in the total fungal count from 4.4 ×10^{2} to 5.9×10^{2} CFU/ml from day1 to 7 and a decrease from 5.9 ×10^{2}to0 CFU/ml from days 7 to after distillation for the setup that involved Aspergillus niger. There was also an increase in the total yeast count from 1.6 ×10^{2} to 2.9 ×10^{2} from days 1 to 7 and a decrease from 2.9 ×10^{2} to 0 from days 7 to after distillation for Saccharomyces cerevisiae. This study shows that alcohol can be successfully produced from cassava using Aspergillus niger and Saccharomyces cerevisiae.

Keywords: Ethanol production, Fermentation, Cassava substrate, *Aspergillus niger*, *Saccharomyces cerevisiae*.

INTRODUCTION

Cassava (*Manihot esculenta*) is one of the major root crops that provide the major part of the daily calorie needs of people in the tropics. It is one of the major root crops that grows abundantly in the region compared with grains. Unlike grains, cassava, like other root and tuber crops have high moisture content mainly responsible for poor storage of the product and utilization potential with an estimated post-harvest life of less than 72 hours and post-harvest loss of about 23% for freshly harvested roots (Omemu *et al*., 2005).

Bioethanol is a biochemical liquid obtained by fermentation of sugar through the catalysis of microorganisms followed by a distillation process (Anuj *et al*., 2007; Adelekan, 2010). It is a renewable energy resource produced by

fermentation process and also can be produced chemically by reacting ethylene with steam (Anuj *et al*., 2007). Bioethanol produced from biomass is a fuel that does not exhibit greenhouse effect (Kroumov *et al*., 2006; Anuj *et al*., 2007; Oyeleke *et al*., 2012). The main sources of sugar required to produce ethanol comes from energy crops such as maize, cassava and cassava products, wheat crops, waste straw, guinea corn husk, rice husk, miller husk, sawdust, sorghum plant, sugar cane, sweet potato etc. (Kim and Dele, 2005; Balat and Balat, 2008).

Fermentation is an important metabolic process that enhances the release of energy from sugar or other organic molecules, which do not require oxygen or an electron transport system and uses an organic molecule as the final electron acceptor (Tortora *et al*., 2010). During fermentation, it is important to monitor and control the process because factors such as temperature, pH, sugar content and microorganisms can affect the overall outcome of the desired end-product.

The quest by many countries for energy independence as well as the widespread awareness of the need to reduce greenhouse gas emissions have heightened the search for alternative energy sources (Farrell *et al*., 2006). Biofuels are expected to reduce dependence on imported petroleum products with associated political and economic vulnerability, reduce greenhouse emissions and other pollutants and revitalize the economy by increasing demand and prices for agricultural products (Balat and Balat, 2008). There is an increasing demand for bioethanol as alternative source of energy in Nigeria, as she currently depends on the importation of ethanol to meet local demand.

Studies reported by Ado *et al*., (2009), Mustafa *et al*. (2019), Umeuzuegbu (2022) have shown *Aspergillus niger* and *Saccharomyces cerevisiae* possess high potential to convert cassava substrates into bioethanol. Hence, this study is aimed at contributing to ongoing effort to produce bioethanol using cassava as a substrate fermented *Aspergillus niger* and *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Sample Collection

Freshly harvested cassava tubers were obtained from Otuoke Community market in Otuoke, Ogbia Local Government Area in Bayelsa State, Nigeria. The tubers were taken to the Microbiology laboratory, Federal University Otuoke for further analysis. The tubers were washed properly and de-skinned. The edible part of the cassava tubers was sliced into small size using a sterile knife and then sun-dried to reduce the moisture content to about 10–12 %. The dried cassava slices were ground using a grinding machine to make flour, and the flour was mixed with water in the ratio of 1:5 to form a slurry. The slurry was allowed to undergo saccharification by acid-enzyme hydrolysis by treatment with ammonium chloride and amylase enzyme. *Saccharomyces cerevisiae* isolated from the cassava flour and *Aspergillus niger* isolated from soil samples were used for this study. The fungal isolates were characterized and tentatively identified using morphological and biochemical methods described by Oyeleke *et al*. (2012).

Production of Bioethanol

The fermentation of cassava for bioethanol was carried out along with simultaneous saccharification and fermentation process (SSF) as described by Kroumov *et al*. (2006) and Oghgren *et al*. (2006). The fermentation was carried out in two (2) setups: One was fermented by *Aspergillus niger* and the other *Saccharomyces cerevisiae*. The process of fermentation lasted for a period of fourteen (14) days and aliquots of the samples were taken at 48hrs intervals to monitor the process.

Determination of fungal count of the bioethanol

The samples were serially diluted and the dilution factor 10^{-2} was inoculated on potato dextrose agar and incubated for 72 hrs. This

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procedure was done in duplicate after which colonies were identified, counted and the values recorded. The procedure was carried out at an interval of two (2) days.

Physicochemical analysis of the bioethanol

The analyses of the physicochemical parameters (temperature, pH, specific gravity, alcohol content) was monitored for fourteen (14) days at an interval of two (2) days.

Determination of Temperature

Ten (10) ml of the cassava slurry was collected from the fermenter and placed into a sterile 50ml beaker. A laboratory mercury bulb thermometer was inserted into the beaker and the readings of the temperature in the course of the fermentation was recorded in ^{o}C.

Determination of pH

Ten (10) ml of the cassava slurry was collected from the fermenter and placed into a sterile beaker and a digital pH meter calibrated with standard buffers (pH 4 and 7) was inserted into the beaker to measure the pH (Ochai and Kolhatkar, 2008).

Determination of specific gravity

Fifty (50) ml specific gravity bottle was cleaned with distilled water, oven dried and allowed to cool. The weight of the cool, dried and empty bottle was recorded as W_1. The dried bottle was filled with deionized water and weighed (W_2). The bottle was emptied and cleaned twice using 5 ml of the cassava slurry and thereafter the bottle was filled to the brim with the cassava slurry and weighed (W_3). The specific gravity was calculated as:

Specific gravity S.G = W_3-W_1/W_2-W_1

Where S = weight of the volume of sample (W_3-W_1)

W= weight of volume of water (W_2-W_1) (AOAC, 2003).

Determination of alcohol content

Ten (10) ml of the cassava slurry was collected from the fermenter and placed into a sterile beaker. An alcohol meter was inserted into the beaker and the alcohol content was measured in percentage (%).

Recovery of ethanol

At the end of the fermentation process, the ethanol produced was recovered using the procedure described by Oyeleke *et al*. (2012). The distillation was carried out using a distillation set up. The fermented liquid was transferred into a round bottom flask and placed on a heating mantle fixed to a distillation column enclosed to a running tap water. Another flask was fixed to the other end of the distillation column to collect the distillate at 78^{o}Cwhich is the standard temperature for ethanol production.

Statistical analysis

The data obtained were statistically analyzed using T-test and analysis of variance (ANOVA).

RESULTS

Fig1 shows the total fungal count (CFU/ml) during and after the fermentation. The process was monitored and controlled at an interval of two (2) days and after distillation. The result shows that there was an increase in the microbial count from day1 to 7 and a decrease from day 7 to 14 (after distillation). T-test revealed that there was a significant difference between the count for *Aspergillus niger* and *Saccharomyces cerevisiae* ($P \leq 0.05$)

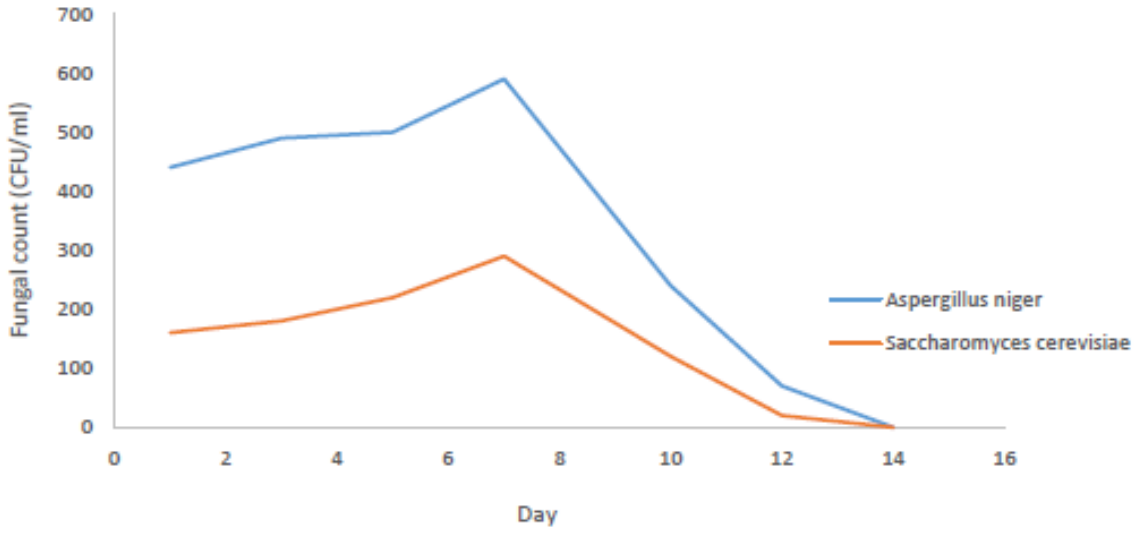

Fig 1: Mean fungal count (CFU/ml) of bioethanol during fermentation and distillation of the product

Figs 2, 3, 4 and 5 shows the results of physicochemical analyses monitored during the fermentation of cassava to ethanol by *Aspergillus niger* and *Saccharomyces cerevisiae*. The result shows that there was a gradual decrease in the pH in the course of the fermentation for the two set ups fermented by *Aspergillus niger* and *Saccharomyces cerevisiae*. There was also a decrease in the specific gravity which probably has a relationship with the increase in alcohol content reported. Statistically, there was no significant difference in temperature and pH of the setup that involved *Aspergillus niger* as fermentation progressed. There was a significant difference in the specific gravity and alcohol content of the setup that involved *Aspergillus niger* as fermentation progressed ($P \leq 0.05$).

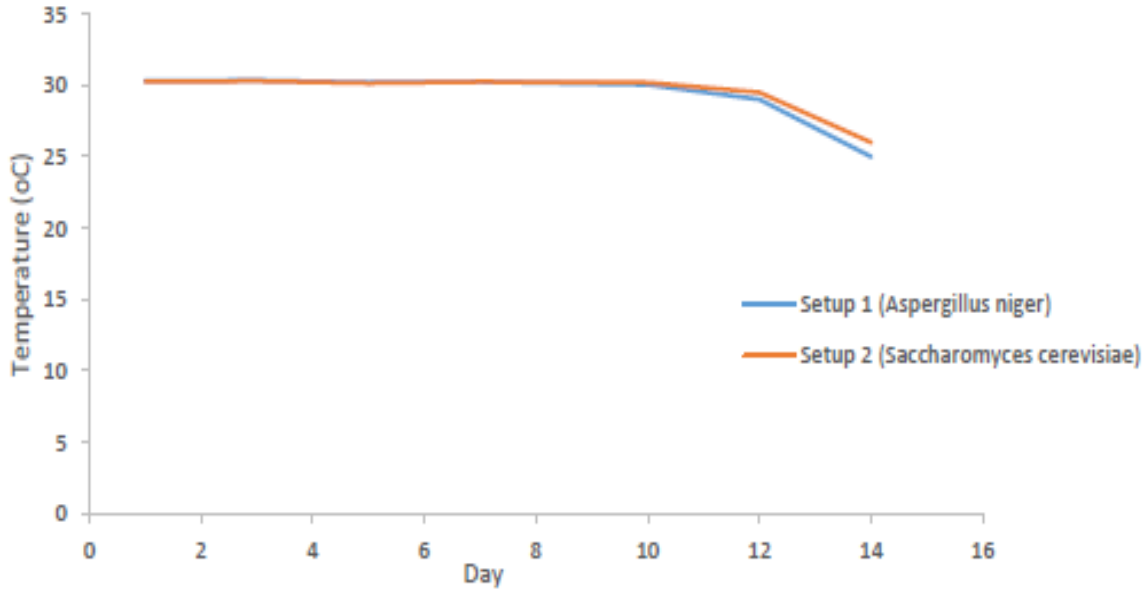

Fig 2: Changes in temperature of bioethanol during fermentation and distillation of the product

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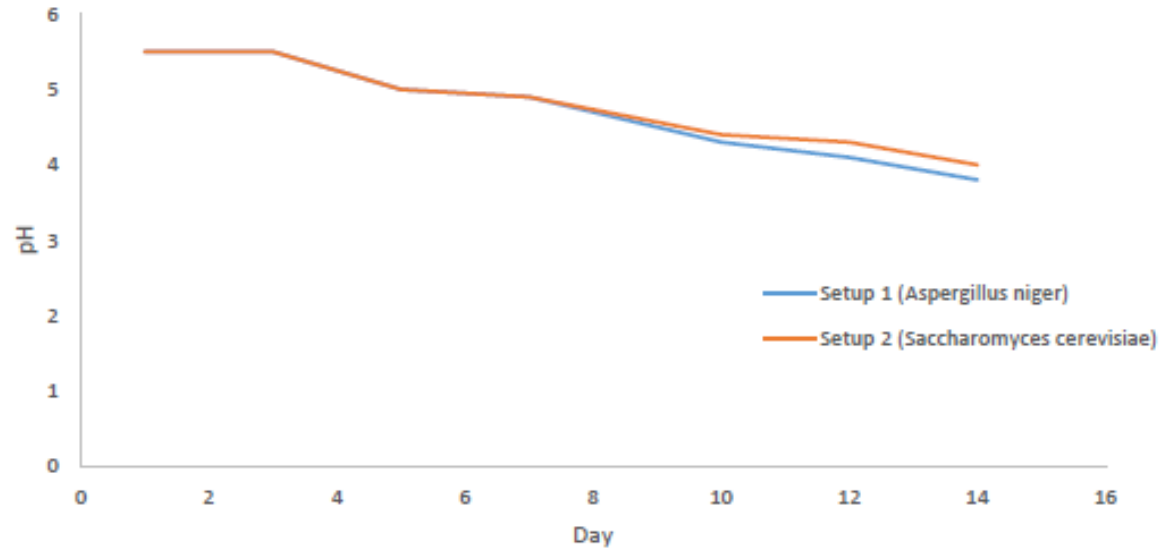

Fig 3: Changes in pH of bioethanol during fermentation and distillation of the product

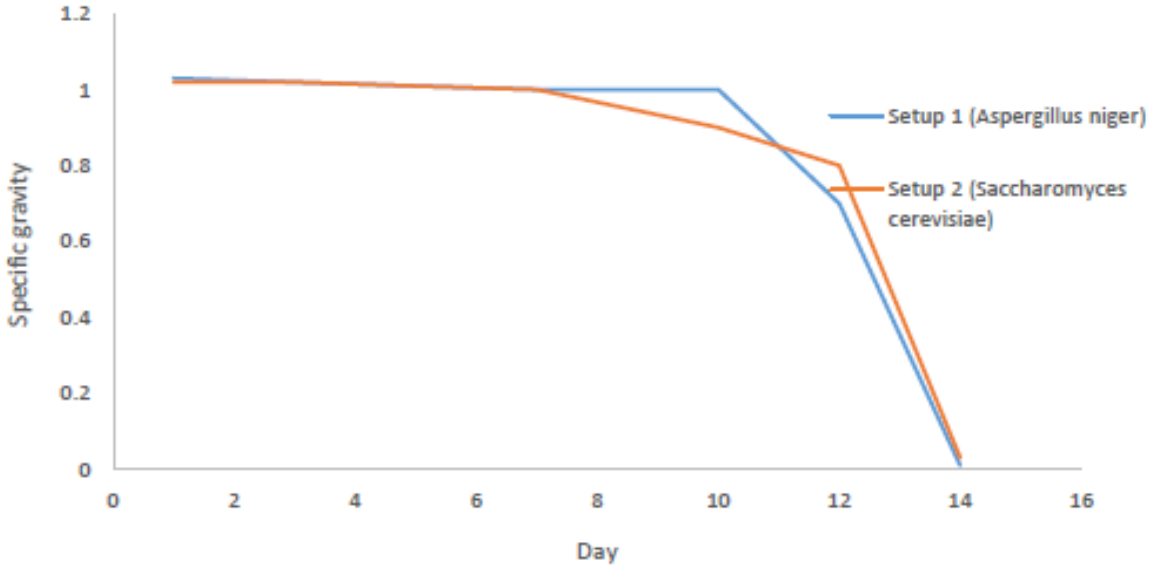

Fig 4: Changes in specific gravity of bioethanol during fermentation and distillation of the product

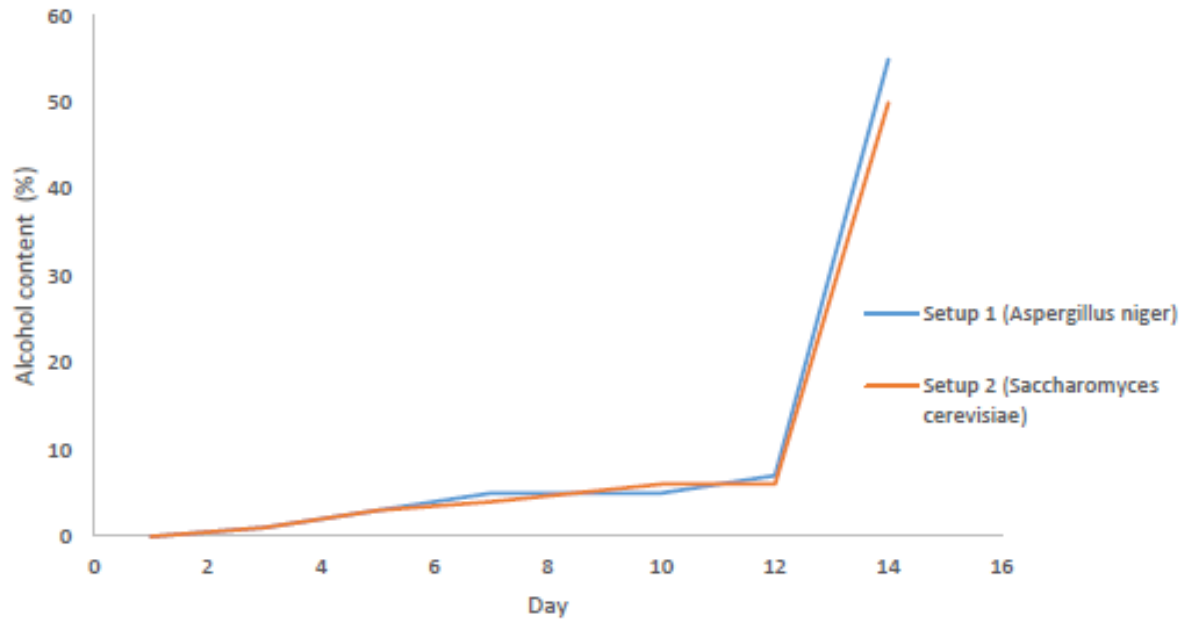

Fig 5: Changes in alcohol content of bioethanol during fermentation and distillation of the product

DISCUSSION

Microbiological Analyses

The fermentation was carried under aseptic conditions in order to obtain a high-quality fermentation yield. The result obtained from this showed that between day 1 to 7, there was a gradual increase in the fungal count from 4.4 x 10^2 CFU/ml to 5.9 x 10^2 CFU/ml for the setup that involved *Aspergillus niger* and 1.6 x 10^2 CFU/ml to 2.9 x 10^2 CFU/ml for *Saccharomyces cerevisiae*. The increase in population of yeast and fungal cells could be attributed to the effective utilization of the sugar. Beginning from day 7 of the fermentation till ethanol distillation was carried out, there was a gradual decrease in the fungal count from 5.9 x 10^2 CFU/ml to 0 for *Aspergillus niger* and 2.9 x 10^2 CFU/ml to 0 for *Saccharomyces cerevisiae*. The reduction in population of yeast and fungal cells could be attributed to the decline in the sugar content due to effective utilization of the sugar by fermenting organisms. This in turn led to an increase in the alcohol content which also affected the growth rate of the fermenting organisms. This report is not in agreement with the findings of Obueh and Ikenebomeh (2014) which recorded 2.62 ×10^5 CFU/ml before fermentation and 2.10 × 10^5 CFU/ml after fermentation.

Physicochemical Analyses

This study revealed that fluctuations in temperature from 25.0ºC to 30.35ºCoccurred during the period of the fermentation. This could be attributed to biochemical changes that occurred during metabolism of the substrate by the fermenting organisms. This temperature range is in accordance with the findings by Ado *et al*. (2009). The researchers reported an increase in temperature from 26.3ºC to 27.9ºC during fermentation of cassava starch to ethanol by co-cultures of *Aspergillus niger*and *Saccharomyces cerevisiae*. The optimum temperature at which the highest quantity of ethanol yield was reported in the setup that involved *Aspergillus niger*and *Saccharomyces cerevisiae* was 25.0 and 26.0

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^{o}C respectively. This was contrary to the findings by Mustafa *et al*. (2019), who recorded an optimum temperature of 28.0^{o}Cduring bioethanol production from cassava waste peels using *Aspergillus niger* and *Saccharomyces cerevisiae*. It was also contrary to the findings by Ona *et al*. (2018), who recorded a temperature of 35.0 ^{o}C during the production of bioethanol from cassava using *Zygomonas morabilis*. This study also revealed that there was a continuous decrease in the pH values during fermentation. The pH decreased from 5.50 to 3.80 and from 5.50 to 4.00 for the set ups that involved *Aspergillus niger* and *Saccharomyces* cerevisiae, respectively from day 1 till after distillation. This report could be attributed to acidification of the medium during fermentation. This is in accordance with the findings by Ado *et al*. (2009) who recorded a decrease in pH from 6.0 to 4.7. The optimum pH with high ethanol yield was 3.80 and 4.00 for the setup that involved *Aspergillus niger* and *Saccharomyces cerevisiae,* respectively. It is in accordance with the findings reported by Mustafa *et al*. (2019) who recorded an optimum pH of 4.55 but, contrary to the findings by Ona *et al*. (2018) who reported optimum pH of 6.0. There exists a correlation between specific gravity and alcohol content. The higher the specific gravity, the lower the alcohol content and the lower the specific gravity, the higher the alcohol content. In the course of the fermentation, there was a decrease in the specific gravity from 1.03 to 0.01 and from 1.02 to 0.03 for the set ups that involved *Aspergillus niger* and *Saccharomyces* cerevisiae, respectively from day 1 till after distillation. The decrease in specific gravity could account for the increase in the alcohol content from 0% to 55.0% and 0% to 50% for the setup that involved *Aspergillus niger* and *Saccharomyces cerevisiae* respectively. This is in agreement with the findings by Ona *et al*. (2018) who reported 63.0% alcohol but it is contrary to the findings reported by Ado *et al*. (2009) and Mustafa *et al*. (2019) who reported ethanol yield of 34.0% and 37.0%, respectively.

CONCLUSION

This study has shown that ethanol can be produced from cassava with a high ethanol content. The production of ethanol from cassava is a means of utilizing locally grown agricultural products to produce bioethanol. It is recommended that further studies we carried out on the utilization of other agricultural products that are readily available in Nigeria.

Competing Interests

The authors have declared that no competing interests exists.

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