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# Effect of inoculum size on solid state fermentation of plantain (*Musa paradisiacea*) by the fungus *Rhizopus oligosporus*

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## Abstract

*Solid state fermentation (SSF) is a vital processing method used for the reduction of postharvest losses, generation of value-added products, and improvement of quality attributes of foods. In this study, the effect of inoculum size on solid state fermentation of plantain by Rhizopus oligosporus was investigated. The results revealed that the solid state fermentation of plantain by R. oligosporus at various inoculum sizes (0 - 30%) significantly ($p < 0.05$) reduced pH and increased the a-amylase activity, glucose and soluble proteins levels of plantain. Fermentation markedly improved the availability of glucose levels and soluble proteins of plantain. It not only improved its nutritional value but also suggested that plantain is a cheap and readily available substrate that can be used in a-amylase production for various industrial applications.*

**Keywords:** Amylase, solid-state fermentation, *Musa paradisiacea, Rhizopus oligosporus*.

## INTRODUCTION

Fermentation is an important microbial processing method used for reducing postharvest losses and improvement of food quality attributes (Latifian *et al*., 2007; Aganbi *et al*., 2020). Among the fermentation techniques, solid state fermentation (SSF) involves the fermentation of solids in the near absence or absence of free water but with sufficient moisture to support the growth and metabolism of microbes (Sabu *et al*., 2006; Egbune *et al*., 2022). In SSF processes, the solid substrate serves as medium and supplies all the nutrients needed for the microbial culture growing in it (Sadh *et al*., 2018). Filamentous fungus, such as *Rhizopus oligosporus*, is less typically utilized in solid state fermentation for food production. *R. oligosporus* is employed in SSF to manufacture food and feed since it does not create any hazardous compounds and is easy to cultivate owing to its lack of pathogenic

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potential (Tonukari *et al*., 2016; Aganbi *et al*., 2020; Egbune *et al*., 2021a). This process is known to greatly enhance the nutrient content of foods through the biosynthesis of several vitamins, essential amino acids, and proteins; improve protein quality, fibre digestibility, and micronutrient bioavailability; as well as degrade anti-nutritional factors (Steinkraus *et al*., 1983; Latifian *et al*., 2007). Production of extracellular hydrolytic enzymes and other metabolites in high amounts using this technique has gained much attention in the past two decades (Krishna and Nokes, 2001; Egbune et al., 2023).

Plantain (*Musa paradisiacea*) is a major group of banana plant varieties. Its production is not only an active and dynamic industry that is central to the wellbeing of many people, but also a crucial part of the economy in the tropical and subtropical regions of the world (FAO, 2014; Mebratie *et al*., 2015). Plantain is highly perishable because of its high moisture contents and metabolic activity which persist after harvest (Falade and Olugbuyi, 2010; Mebratie *et al*., 2015). The postharvest losses are significant especially in developing nations where there is poor or lack of storage facilities or inapt technologies for food processing (Falade and Olugbuyi, 2010; Mebratie *et al*., 2015). Thus it is mostly processed into plantain flour (Oluwalana *et al*., 2011). Traditionally, plantain flour is mainly used for stiff dough, baked products (Olaoye *et al*., 2006; Oluwalana *et al*., 2011) and as a component of baby foods (Aurore *et al*., 2009; Mohapatra *et al*., 2009).

It is well established that processing conditions such as drying and milling applied to plantain would affect its functional and physicochemical properties (Maskan, 2000; Falade and Olugbuyi, 2010). Thus, this study was aimed at determining the effects of inoculum size on SSF of plantain (*M. paradisiacea*) by the fungus *R. oligosporus*. The fungus, at different inoculum sizes, was employed in the SSF of plantain to establish the level (inoculum size) that generated maximum amounts of the analyzed value-added products. This will promote the use of plants such as plantain for production of useful and different value-added products including biofuels, chemicals and cheap energy sources for fermentation, improved animal feeds, and human nutrients.

## MATERIALS AND METHODS

### Plant, starter organism and reagents

Unripe plantains (*M. paradisiacea*) were collected at Oviorie-Ovu, Delta State, Nigeria. *R. oligosporus* strains (produced by PT Aneka Fermentasi Industri, Bandung-Indonesia) were procured from Harmony Pathological Laboratory Ltd., Songhai, Delta State, Nigeria. Distilled water was used while all the chemicals and reagents used were of analytical grade.

### Solid state fermentation process

The unripe plantain was peeled, cut into small sizes and then air dried under shade for about one week. The dried chips were further reduced to powder form using an electric grinding machine (SM-1 Retsch GmbH 5667 HAAN, West Germany). Different inoculum sizes (5, 10, 15, 20, 25 and 30%) of *R. oligosporus* were homogenized in 15 mL of acetate buffer (50 mM; pH 6.0) in seven different labeled Petri-dishes and thereafter mixed thoroughly with 7.0 g of the ground plantain flour. A control (0%) was prepared following the aforementioned procedure but was devoid of any presence of mold cells. All Petri-dishes were then covered and the plantain flour was allowed to ferment at room temperature for 72 hours. After the fermentation period, 6.0 g of the mixture was taken from each of the Petri dishes, mixed with distilled water (40.0 mL) and then homogenized using a mortar and pestle. The homogenate (10.0 mL) was measured into a test tube, centrifuged for 10 min, and

the supernatant obtained was stored in a sterile universal container at room temperature (25°C) as sample for the various biochemical assays. The pH was measured using a Metrohm 620-pH meter equipped with a glass electrode.

**Determination of α-amylase activity**

Alpha-amylase activity was determined following the modified method described by Nouadri *et al*. (2010), with slight modifications. A mixture of soluble starch (0.5 mL; 1.0% w/v) in phosphate buffer (0.1 M; pH 6.5) and the sample (0.5 mL) were measured into series of labeled test tubes. Also, standard and blank test tubes were prepared using maltose solution (in the range of 0.0 to 2.0 mg/mL) and distilled water, respectively in place of the samples. After all the test tubes were incubated at 30°C for 30 min, the reaction was terminated by adding 2.0 mL of 3,5-dinitrosalicylic acid reagent. It was placed in boiling water for colour development for 5 min and cooled to room temperature. 10.0 mL of distilled water was added to each reaction tube and read at 540 nm wavelength using a UV-Vis (ThermoFisher Scientific G10S) spectrophotometer.

With the aid of the maltose standard calibration graph, the amount of maltose liberated in the sample tubes was obtained and used to deduce the amylase activity as shown in Equation 1. This was expressed in unit (U) which is the amount of enzyme that released 1.0 µg of maltose (as reducing sugar equivalent)/mL/min under the assay conditions.

$$\text{Enzyme activity (U)} = \frac{\text{µg of maltose released}}{\text{Volume (ml) of enzyme used} \times \text{incubation time (30 min)}} \quad (1)$$

**Quantitative determination of glucose**

Glucose was determined following the method described in the Randox glucose test kit. Equal volumes (20 µl) of the standard, blank (distilled water) and the various samples were pipetted into a series of labeled test tubes, and then mixed with 2.0 mL of the reconstituted glucose working reagent. The mixtures were allowed to incubate at 25°C for 25 min and read at 500 nm with UV-Vis (ThermoFisher Scientific G10S) spectrophotometer. Glucose concentration was then calculated using the formula (Equation 2):

$$\text{Glucose Conc.}\,(\text{mg/dl}) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard Conc.} \quad (2)$$

**Determination of total soluble proteins**

This was conducted using the standard method described by Gornall *et al*. (1949). Equal volumes of protein standards (ranging in concentration from 0.5 to 10 mg/mL), distilled water and the various samples were measured in labeled test tubes such that the final volume was 0.5 mL. Thereafter, 2.5 mL of Biuret reagent was added to all tubes, vortexed, and allowed to stand for 30 min, and read at 540 nm wavelength using UV-Vis (ThermoFisher Scientific G10S) spectrophotometer. The amount of soluble proteins in the sample was estimated directly from the standard graph of absorbance of the standard (bovine serum albumin) against concentration (mg/mL).

**Statistical analysis**

Microsoft Excel (version 2007) and SPSS PC programme package were used for data processing and analyses. The results from the experimental procedures were analyzed using one-way analysis of variance (ANOVA). The values were reported as Mean ± Standard

deviation of triplicate determinations while comparison was made at 95% confidence level ($p<0.05$).

## RESULTS AND DISCUSSION

Solid state fermentation protocol is a potential technological method used for processing various foods to enhance their nutritional qualities and to generate value-added products (Pandey, 2003; Latifian *et al*., 2007; Aganbi *et al*., 2020; Anigboro *et al*., 2022a). Specifically, this study was aimed at determining the effects of inoculum size on pH, amylase activities, glucose levels and soluble proteins of solid state fermented plantain (*M. paradisiacea*) by the fungus *R. oligosporus*.

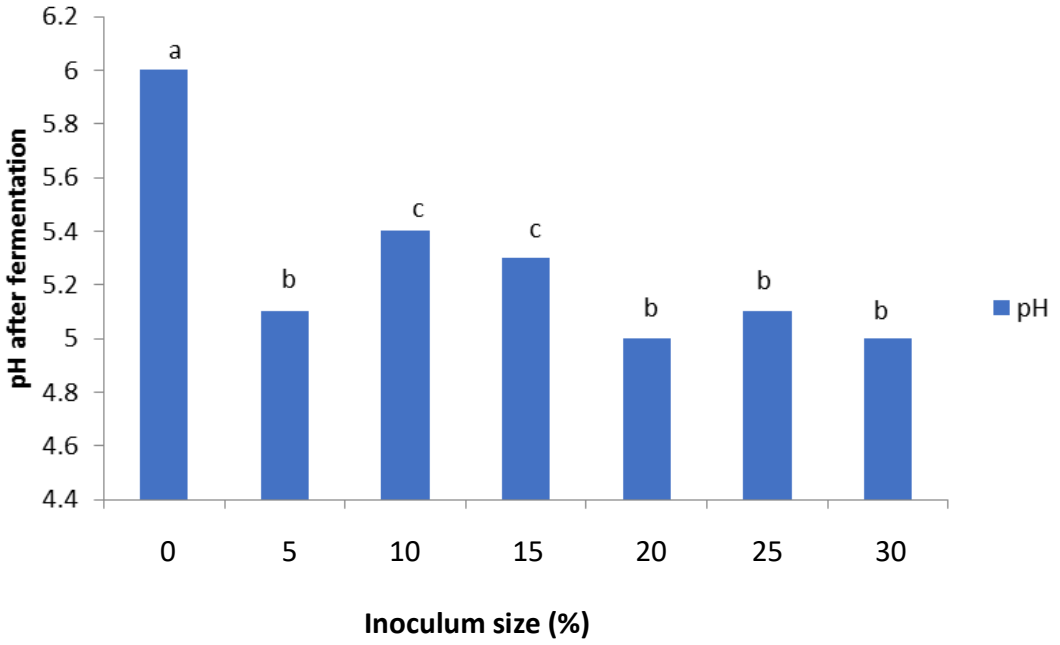


**Figure 1.** pH changes in the fermentation of plantain samples using *R. oligosporus.* The identification of each bar with various letters revealed a substantial difference at $p<0.05$.

The outcome of the effect of inoculum size on the pH changes in the plantain samples fermented with *R. oligosporus* (Figure 1). This shows that the pH of all the fungus fermented plantain was mainly acidic and significantly different ($p<0.05$) from the pH of the unfermented control (which remained unchanged). However, the samples fermented at 10 and 15% inoculum sizes were substantially less acidic than the other fungus fermented samples. It has been shown in a previous study that during fermentation, the pH in the fermenting sample declined with time as a result of acid and alcohol accumulation (Adejuwon *et al*., 2019; Anigboro *et al*., 2022b). Oduah *et al*. (2015) also reported a similar decrease in pH of cassava mash after four days of fermentation. It was attributed to the accumulation of organic acids like lactic and acetic acids in the fermented cassava produced by the activities of *R. oligosporus*.

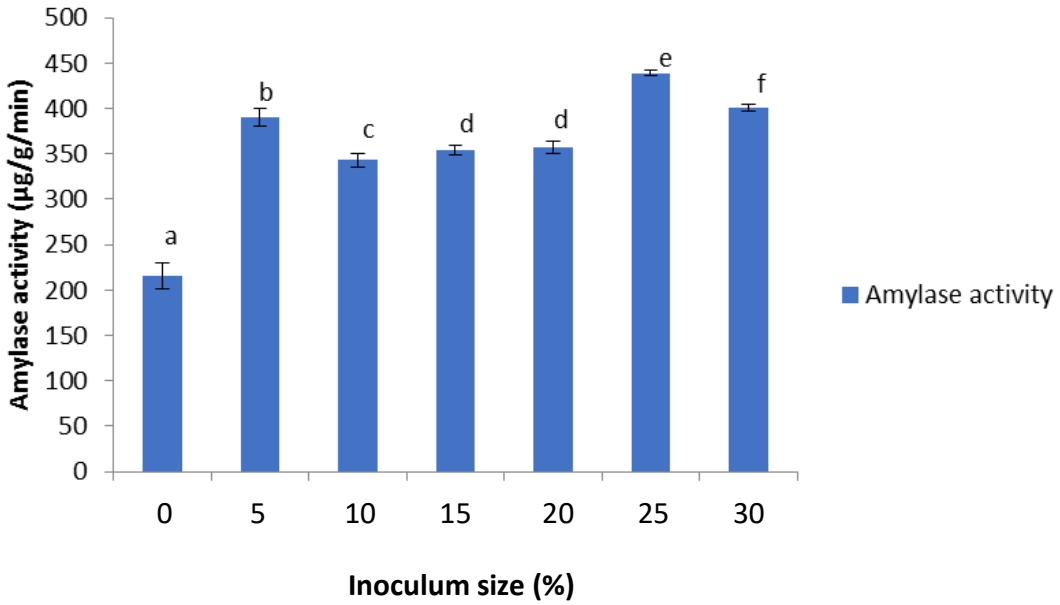


**Figure 2.** Amylase activity at different inoculum size during solid state fermentation of plantain with *R. oligosporus*. Each bar is an expression of mean ± standard deviation; the identification of each bar with various letters revealed a substantial difference at $p<0.05$.

The result of the effect of inoculum size on amylase activity during SSF of plantain by *R. oligosporus* is expressed as µg of maltose released by the enzyme per gram of the sample per minute (Figure 2). This shows that fermentation resulted in a noteworthy increase in amylase activity at all inoculums sizes with highest activity (438.92±3.02 µg/g/min) obtained at 25%. This increase in activity may be due to its secretion by the fungus for degradation of starch to glucose required as substrate for microbial growth. A similar pattern of results was obtained by Saxenal and Singh (2011) in their study on amylase production by SSF of agro-industrial wastes using *Bacillus* species. Also, Saharan *et al.* (2017) and Ojo *et al.* (2022) reported the role of α-amylase and other enzymes in the release of polyphenols and antioxidants during SSF of cereals. As at 2012, α-amylase production contributed about 25 to 30% to the US$2.7 billion world enzyme market (Elmarzugi *et al.*, 2014) and with Nigeria being one of the largest producers of plantain in Africa (FAO, 2006). This finding infers an added value to plantain; a reduced environmental problem; and a source of revenue and diversification for its economy.

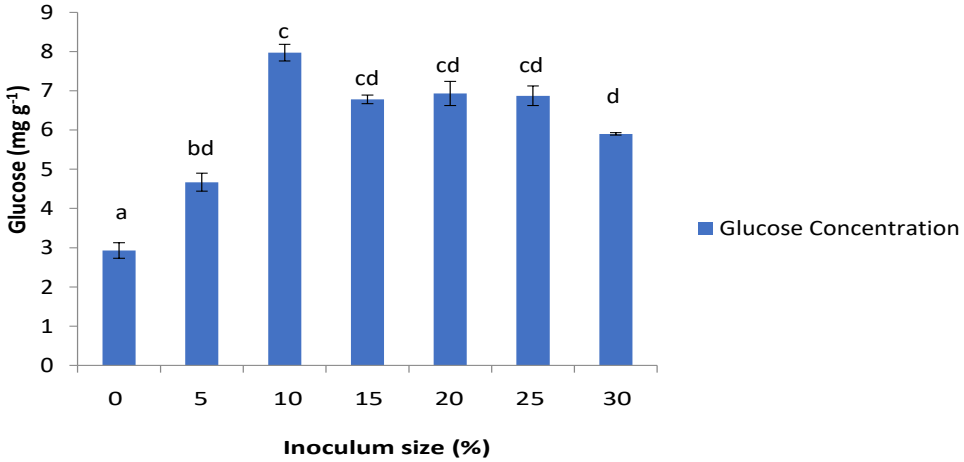


**Figure 3.** Effect of inoculum size on glucose levels in solid state fermented plantain using *R. oligosporus*. Each bar is an expression of mean ± standard deviation; the identification of each bar with various letters revealed a substantial difference at $p<0.05$.

Glucose, which may be obtained from starch degradation, is a major source of carbon required for the sustenance of microbial growth. The results of the glucose assay in this study (Figure 3) showed that glucose concentration remarkably increased at all inoculums sizes with highest level (7.97±0.21 mg $g^{-1}$) obtained at 10%. Similar finding was reported on *R. oligosporus* fermentation of maize (*Zea mays*) offal (Anigboro *et al*., 2020). This may be attributed to the ability of the microorganism to secrete some extracellular enzymes, which degrade crude fibre and complex polysaccharides to glucose during fermentation as carbon source to synthesize rich fungal biomass (Oboh and Akindahunsi, 2003; Egbune *et al*., 2022; Ndego *et al*., 2023).

Solid state fermentation of plantain with *R. oligosporus* resulted in a noticeable increase ($p<0.05$) in soluble protein levels at all inoculum sizes with the highest value (169.70 ± 1.01 mg/g) observed at 20% (Figure 4). The observed increase in protein levels is in consonance with the works of Aganbi *et al*. (2020) and Egbune *et al*. (2021b) who reported that fermentation may be a process through which protein contents and product quality are improved. The increase in protein content after the fermentation could be due to microbial metabolic activities that emanated from the release of bacterial proteins and extracellular enzymes into the samples (Oseni and Ekperigin, 2007; Eromosele *et al*., 2017), and/or increase in microbial cell mass.

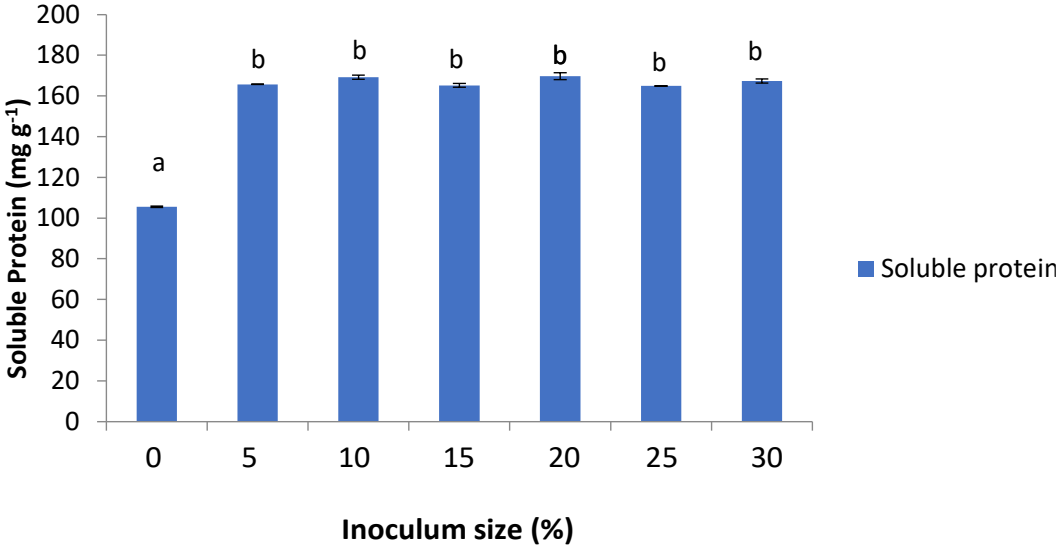


**Figure 4.** Effect of inoculum size on soluble protein levels during solid state fermentation of plantain with *R. oligosporus*. Each bar is an expression of mean ± standard deviation; the identification of each bar with various letters revealed a substantial difference at $p<0.05$.

Overall, the choice of a substrate for SSF process depends on a number of factors ranging from cost, availability to capacity to supply the nutrients for the sustenance of microbial culture growth (Okhonlaye *et al*., 2016). In this study, *R. oligosporus* and plantain were reaffirmed to be suitable microorganism (Aganbi *et al*., 2020) and substrate, respectively for the biotechnological process. This process not only improved the digestibility (via increased α-amylase activity), nutritive functionalities (higher amounts of glucose, proteins), shelf-life, and organoleptic properties (reduction of pH) of the plant, but also enhanced its ability to produce value-added biomolecules.

## CONCLUSION

This study established not only that SSF process improved the nutrition qualities of plantain, but also its suitability as a cheap and readily available substrate. It can be employed by solid state fermentation in α-amylase production generally used for different industrial applications, thus promoting the value of plantain and reducing the amount of this agro industrial waste in the environment.

## REFERENCES

Adejuwon, A. O., Tsygankova, V. A. andObayemi, O. S. (2019). α-Amylase Production Using *Aspergilusvadensis* Isolated from Pulverized Cocoa Seeds. *Life Science Journal*, **16(8):** 64 – 70. DOI:10.7537/marslsj160819.08

Aganbi, E., Anigboro, A. A. and Tonukari, N. J. (2020). Changes in glucose, amylase and soluble proteins levels in solid state fermented yam (*Dioscorea*spp.) peels by *Rhizopusoligosporus*. *Nigerian Journal of Science and Environment*, **18(1):** 161 – 167.

Anigboro, A. A., Aganbi, E. and Tonukari, N. J. (2020). Solid State Fermentation of Maize (*Zea mays*) Offal by *Rhizopusoligosporus*under Acidic and Basic Conditions. *Journal of Scientific Research*, **12 (4):** 751-756.

Anigboro, A. A., Egbune, E. O., Akeghware, O., Evie, P., Samofordu, A. A. and Tonukari, N. J. (2022b). Biochemical parameters of solid-state fermented cocoyam (Colocasia esculenta) using Rhizopus oligosporus at different inoculum sizes. Nigerian Journal of Biotechnology, 39(1), 68-74.

Anigboro, A. A., Egbune, E. O., Akeghware, O., Ovowa, F. O., Anyanwu, N. B., Chebene, C., Ogbeyi, C. and Tonukari, N. J. (2022a). Changes in biochemical parameters during the solid- state fermentation of pineapple (Ananas comosus I. Merr) peels by Rhizopus oligosporus. Bayero Journal of Pure and Applied Sciences, 15(1), 74-79.

Aurore, G., Parfait, B. and Fahrasmane, L. (2009).Bananas, raw materials for making processed food products. *Trends in Food Science and Technology*, **20(2):** 78 – 91. DOI:10.1016/j.tifs.2008.10.003

Egbune, E. O., Aganbi, E., Anigboro, A. A., Ezedom, T., Onojakpor, O., Amata, A. I. and Tonukari, N. J. (2023). Biochemical characterization of solid-state fermented cassava roots (Manihot esculenta Crantz) and its application in broiler feed formulation. World Journal of Microbiology and Biotechnology, 39(2), 1-12.

Egbune E. O., Orhonigbe I., Adheigu R. O., Oniyan U. P. and Tonukari N. J. (2021a). Effect of inoculum size on solid state fermentation of pearl millet (Pennisetum glaucum) by *Rhizopus oligosporus*. *Nigerian Journal of Science and Environment* 20 (1): 1-9.

Egbune, E. O, Eze, E, Edwards, R. A., Ezedom, T. and Tonukari NJ, (2021b). Enhancement of the nutritional value of elephant grass (Pennisetum purpureum Schum.) for use as animal feeds and for xylanase production. Nigerian Journal of Science and Environment, 19(2).

Egbune, E. O., Avwioroko, O. J., Anigboro A. A., Aganbi, E., Amata, A., Tonukari, N. J. (2022). Characterization of a surfactant-stable α-amylase produced by solid-state fermentation of cassava (Manihot esculenta Crantz) tubers using Rhizopus oligosporus: Kinetics, thermal inactivation thermodynamics and potential application in laundry industries. Biocatalysis and Agricultural Biotechnology 39. 102290.

Elmarzugi, N., Hesham, E., Mariani, A., Rosnani, H., Azilz, A., Elsayed, E., Nor, O. and Salama, M. (2014). Amylase Economic and Application Value. *World Journal of Pharmaceutical Research*,**3:** 4890 – 4906.

Eromosele, O., Ojokoh A. O., Ekundayo, O. A, Ezem L. and Chukwudum, A. A., (2017). Effect of Fermentation on the Proximate Composition of Ripe and Unripe Plantain Flour.*Journal of Advances in Microbiology*,**2 (3):** 1 – 10.DOI: 10.9734/JAMB/2017/31303

Falade, K. O. and Olugbuyi, A. O. (2010). Effects of maturity and drying method on the physico-chemical and reconstitution properties of plantain flour.*International Journal of Food Science and Technology*, **45(1):** 170-178. https://doi.org/10.1111/j.1365-2621.2009.02118.x

FAO (2014). FAO STAT base.Food and Agriculture Organization of the UnitedNations, Rome Italy. Available at:http://faostat.fao.org (accessed Dec, 2015).

Gornall, A.G., Bardawill, C.J.and David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, **177(2):**751– 766.

Latifian, M., Hamidi-Esfaham, Z. and Barzegar, M. (2007). Evaluation of culture conditions for cellulose production by two *Trichodermareesei* mutants under solid-state fermentation conditions. *Bioresource Technology*, **98:** 3634 – 3637. DOI:10.1016/j.biortech.2006.11.019

Maskan, M. (2000). Microwave/air and microwave finish drying of banana. *Journal of Food Engineering*, **44:** 71 – 78. DOI:10.1016/S0260-8774(99)00167-3

Mebratie, M. A., Haji, J., Woldetsadik, K. and Ayalew, A. (2015).Determinants ofpostharvest banana loss in the marketing chain of central Ethiopia. *Food Science and Quality Management*, **37:** 52-63.

Mohapatra, D., Mishra, S. and Meda, V. (2009). Plantains and their postharvest uses:An overview. *Stewart Postharvest Review*, **5(5):** 1-11. DOI:10.2212/spr.2009.5.4

Ndego, A., Ezedom, T., Egbune, E. O. and Tonukari, N. (2023). Biochemical characterization of solid state fermented maize cob (Zea mays) using Rhizopus oligosporusand its application in poultry feed production. International journal of recycling organic waste in agriculture, 12(2), 235-246.

Nouadri, T., Meraihi, Z., Shahrazed, D. D. and Leila, B. (2010). Purification and characterization of the α-amylase isolated from *Penicilliumcamembert*iPL21. *African Journal of Biochemical Research,* **4 (6):**155-162.

Oboh, G. and Akindahunsi, A. (2003). Biochemical changes in cassava products (flour andgari) subjected to *Saccharomycescerevisae*. Solid Media Fermentation. *Food Chemistry*, **82:** 599 – 602. DOI:10.1016/S0308-8146(03)00016-5.

Oduah, N.O., Adepoju, P.A., Longe, O., Elemo, G.N. and Oke, O.V. (2015). Effects of fermentation on the quality and composition of cassava mash (Gari). *International Journal of Food Nutrition and Safety*, **6(1):** 30 – 41.

Ojo, I., Apiamu, A., Egbune, E. O. and Tonukari, N. J. (2022). Biochemical Characterization of Solid-State Fermented Cassava Stem (Manihot esculenta Crantz-MEC) and Its Application in Poultry Feed Formulation. Applied Biochemistry and Biotechnology, 194(6), 2620-2631.

Ojokoh, A., Foluke, O. and Rattanachaikunsopon, P. (2016). Fermentation of Cassava (*Manihotesculenta*) and Ripe Plantain Peels (*Musa paradisiaca*) in the Production of Animal Feed. *Journal of Advances in Microbiology*, **1:** 1 – 15. DOI:10.9734/JAMB/2016/30354

Olaoye, O., Onilude, A., and Idowu, O. (2006). Quality characteristics of breadproduced from composite flours of wheat, plantain and soybeans. *African Journal of Biotechnology*, **5(11):** 1102-1106.

Oluwalana, I.,Oluwamukomi, M., Fagbemi, T. and Oluwafemi, G. (2011).Effects oftemperature and period of blanching on the pasting and functional propertiesof

plantain (*Musaparasidiaca*) flour. *Journal of Stored Products andPostharvest Research*, **2(8):** 164-169.

Oseni, O. and Ekperigin, M. (2007). Studies on Biochemical Changes In Maize Wastes Fermented With *Aspergillus Niger*. *Biokemistri*, **19 (2):** 19. DOI:10.4314/biokem.v19i2.56428

Pandey, A., Soccol, C. R. and Mitchell, D. (2003). New developments in solid-state fermentation: I-Bioprocess and products. *Process Biochemistry*, **35 (10):**1153– 1169. DOI:10.1016/S0032-9592(00)00152-7

Sabu, A., Augur, C., Swati, C. and Pandey, A. (2006). Tannase production by *Lactobacillus* sp. ASR-S1 under solid-state fermentation. *Process Biochemistry*, **41(3):** 575 – 580. https://doi.org/10.1016/j.procbio.2005.05.011

Sadh, K. P., Duhan, S., and Duhan, S. J. (2018). Agro-industrial wastes and their utilization using solid state fermentation: a review.*Bioresources and Bioprocessing*,**5:**1. https://doi.org/10.1186/s40643-017-0187-z

Saharan, P., Sadh, P. K., and Duhan, J. S. (2017). Comparative assessment of effect of fermentation on phenolics, favonoids and free radical scavenging activity of commonly used cereals. Biocatalyst and Agricultural Biotechnology, 12: 236–240. DOI:10.1016/j.bcab.2017.10.013

Saxena, R. and Singh, R. (2011). Amylase production by solid-state fermentation of agro-industrial wastes using Bacillus sp. *Brazilian Journal of Microbiology*, **42 (4):**1334 – 1342. DOI:10.1590/S1517-838220110004000014

Steinkraus, K. H., Cullen, R. E., Pederson, C. S., Nellis, L. F. and Gavitt, B. K. (1983). Indonesian tempeh and related fermentations. In: Handbook of indigenous fermented foods. Steinkraus, K. H.*et al*. (Eds.), New York, USA, pp. 1-94.

Tonukari, N. J., Egbune, E. O., Avwioroko, O. J., Aganbi, E., Orororo, O. C. and Anigboro, A. A. (2016). A novel pig feed formulation containing Aspergillus niger CSA35 pretreated-cassava peels and its effect on growth and selected biochemical parameters of pigs. *African Journal of Biotechnology*. 1519 776-785.