

Biochemical parameters of solid-state fermented cocoyam (*Colocasia esculenta*) using *Rhizopus oligosporus* at different inoculum sizes

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Abstract

In this study, the effect of different inoculum sizes of *Rhizopus oligosporus* on solid-state fermented (SSF) cocoyam (*Colocasia esculenta*) was evaluated. The results of the study show that the samples fermented with *R. oligosporus* at different inoculum sizes (5-30%) had statistically higher activity of α -amylase, as well as higher levels of proteins and glucose, but significantly reduced pH and reducing sugar contents ($p < 0.05$) compared to the control (0%) after 72 h of fermentation. The optimal levels of amylase activity, glucose and soluble proteins were observed in the samples fermented using inoculum sizes between 10 and 20%. The study revealed that solid-state fermentation of cocoyam enhanced its shelf life, nutritive value and bioavailability of nutrients. This suggests that it is not only an excellent component for human and animal feed production, but also a suitable substrate for industrial production of important biomolecules.

Key words: Inoculum sizes, soluble protein, fermentation, *Colocasia esculenta*, *Rhizopus oligosporus*.

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Introduction

Solid-state fermentation (SSF) process involves the cultivation of microbes in an environment that is characterized by very low or absence of free moisture content (Soccol et al., 2017). Evidently, it is a very promising biotechnological operation that has several biological advantages including wastes reuse. This is because it allows the use of several solid agro-industrial wastes as substrate and/or energy source in their natural form and enhances the management of such solid waste, with very low wastewater generation (Anigboro et al., 2014; Sadh et al., 2018). This process provides the suitable and optimum conditions that resemble the natural environment of most microbes such as fungi and mold that are employed. It requires little or no sterilization energy (because of the very low water activity). Also, it is relatively resistant and less prone to microbial contamination and inhibition of the desired microbial processes thus, giving rise to generation of high concentrations of the desired products. Generally, solid state fermentation, like the traditional fermentation technique, has been shown to

improve the nutritional quality of foods through the biosynthesis of essential nutrients, enhance protein quality, and increase fibre digestibility and micronutrient bioavailability as well as detoxification via inactivation of anti-nutritional factors (Abegaz, 2007; Oyarekua, 2013; Morgan and Choct, 2016; Aganbi et al., 2020; Anigboro et al., 2020a).

Cocoyam (*Colocasia esculenta*) is a common herbaceous perennial plant that belongs to the Araceae family and is mainly grown for its edible roots on a relatively small-scale basis (Adegunwa et al., 2011). About three-quarter of the world's cocoyam production is from Africa, with the leading producers being Nigeria and Ghana. Cocoyam is a carbohydrate-rich cash crop and considered as third in Nigeria in terms of importance (Tambong et al., 1997; Nyochembeng and Garton, 1998; Olayiwola et al., 2013), though it is still insufficiently studied and underutilized (Watanabe, 2002). Efforts also need to be geared towards increasing its acceptability among the populace through the adaptation of suitable technologies (such as solid-state fermentation) that have been applied to other tubers (Adejumo and Bamidele, 2012).

In solid-state fermentation processes, the substrate is an ideal one when it is able to provide all the nutrients required for the microorganisms growing in it as well as a housing medium for the microbial cells (Tonukari et al., 2016; Sath et al., 2018). However, researchers have not done any tangible work on the solid-state fermentation of *C. esculenta*, and this has hampered its need for extensive mechanization and nutritional functionalities in Nigeria. Thus, this study is aimed at determining the effect of different inoculum sizes of *Rhizopus oligosporus* on the simultaneous saccharification and fermentation (SFF) of cocoyam (*C. esculenta*). The present investigation will help increase the value of the product for farmers in both local and international market. It will encourage its use in the production of various useful value-added products (including biofuels, chemicals) and will be used as cheap energy sources for fermentation, improved animal feeds, and human nutrients.

Materials and Methods

Plant materials, starter organism and reagents

Cocoyam (*C. esculenta*) and pure culture of the fungus, *R. oligosporus* (PT Aneka Fermentasi Industri, Bandung-Indonesia brand) were obtained from Abraka Market, Abraka, and Harmony Path. Ltd. Laboratory, Sapele, respectively, both in Delta State, Nigeria. Glass-distilled water was used. The reagents used for the biochemical analyses were of analytical standard.

Substrate preparation for solid-state fermentation process

The obtained cocoyam was verified and authenticated at the Plant Biology and Biotechnology Department, University of Benin, Nigeria. The cocoyam was properly washed with tap water to remove dirt and sliced into small pieces. It was allowed to dry under shade for about two weeks (until a constant weight was achieved), ground into powder and stored in sterile containers. The different inoculums (0- 30%) of *R. oligosporus* prepared with acetate buffer (0.05 M; pH 6.0) in Petri dishes were mixed thoroughly with 7 g of the cocoyam powder and left to undergo fermentation at room temperature for 72 h. Six grams of the fermented flour was homogenized using a mortar and pestle and mixed with 40 ml of distilled water. Then, 10 ml of the homogenate was centrifuged at 4000 g for 10 min and the supernatant obtained was stored in a sterile universal container at room temperature for further use as sample in the various biochemical

analyses. The pH of the medium was measured using a pH meter (Metrohm 620 brand).

Estimation of glucose level

Estimation of glucose level was done with the aid of the standard Randox glucose test kit. Briefly, 20 µl of the various samples, the blank and the standard preparations were mixed with 2.0 ml of the glucose working reagent and incubated at room temperature for 25 min. Thereafter, the absorbances of all reaction tubes were read at 500 nm wavelength using the UV-Vis (GENESYS G10S) spectrophotometer. The level of glucose (in mg/dl) in the samples was evaluated using the equation below:

$$\text{Glucose level} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard conc.}$$

Assessment of protein content

The standard protocol described by Gornall et al. (1949) was used for the assessment of protein level in the samples. To 0.5 ml of the various samples, the blank and protein standards [bovine serum albumin (BSA) [0.5 to 10.0 mg/ml] were added to 2.5 ml of Biuret reagent. They were mixed and incubated at room temperature for 30 min; absorbance was read at 540 nm wavelength using the UV-Vis (GENESYS G10S) spectrophotometer. A standard calibration graph obtained from BSA (from the result obtained in the standard tests) was used to evaluate the number of proteins in the samples as an equivalent of the standard protein used.

Evaluation of reducing sugars content

The 3, 5-Dinitrosalicylic acid (DNSA) method of Miller (1959) was employed for the evaluation of reducing sugars content in the samples. Summarily, 1.5 ml of the samples and 1.5 ml of the prepared DNSA reagent were mixed and incubated at 90°C for 15 min followed by addition of potassium sodium tartrate solution (0.5 ml; 40%). These were cooled to room temperature after which absorbance of all reaction tubes was read at 575 nm wavelength using the UV-Vis (GENESYS G10S) spectrophotometer.

Alpha-amylase activity assay

The activity of α-amylase in the samples was assessed using the standard modified method of Nouadri et al.

(2010). The different samples (0.5 ml) were mixed with soluble starch (1% w/v) in phosphate buffer (0.10 M; pH 6.5). Also, blank and standard tests were prepared as described above but using distilled water and different concentrations of maltose solution (0.0 to 2.0 mg/ml) respectively in place of the samples. The test tubes were incubated at 30°C for 30 min. Thereafter, 2.0 ml of DNSA reagent was added and placed in boiling water for colour development for 5 min and cooled to room temperature. Distilled water (10.0 ml) was then added to each test tube and the absorbance read at 540 nm wavelength using a UV-Vis (GENESYS G10S) spectrophotometer. The results of the standard tests were used to plot a standard calibration graph of absorbance against maltose amount. The α -amylase activity was calculated using the equation below,

$$\alpha - \text{Amylase activity (U)} = \frac{\mu\text{g of maltos}}{\text{Volume (in ml) of samp}}$$

Where one unit (U) of α -amylase activity is the amount of the enzyme that produced 1 μg of maltose per ml per min (as reducing sugar equivalent) under the experimental conditions.

Statistical analysis of data

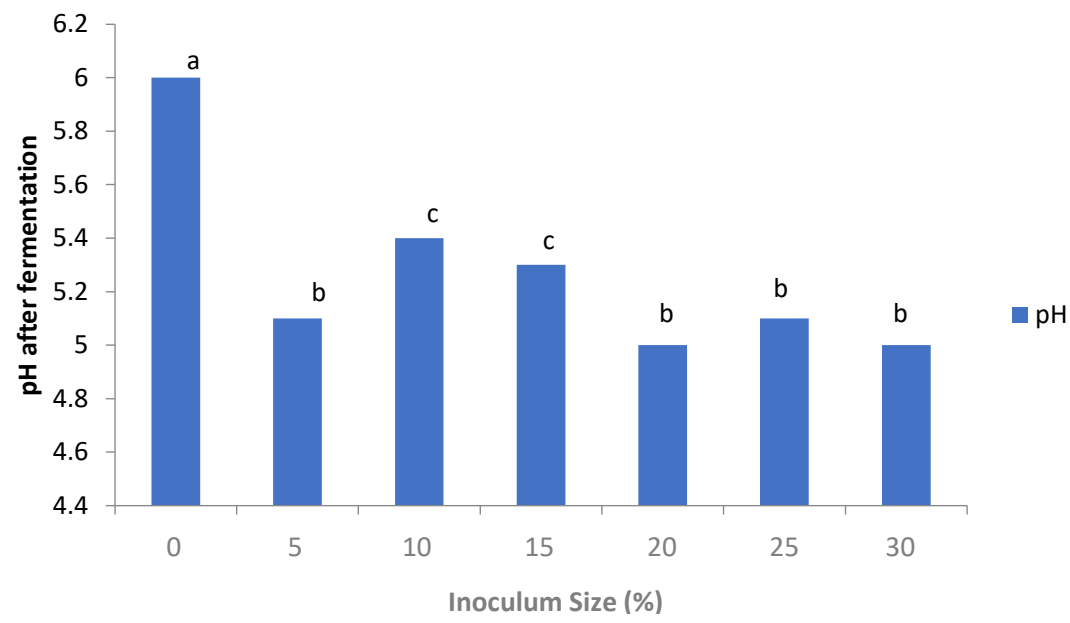


Figure 1: pH of different-inoculum-size fermented *C. esculenta* using *R. oligosporus*. Different letters differ substantially at $p < 0.05$ on each bar, which is a representation of mean SD and represents triple observations ($n=3$).

In this study, the effect of inoculum sizes of *R. oligosporus* on solid-state fermentation of cocoyam (*C. esculenta*) was investigated. Employing this microbial fermentation technique at room temperature for 72 h,

Using the SPSS PC programme software, the data obtained from the various biochemical analyses were subjected to One-way Analysis of Variance (ANOVA), while the Microsoft Excel was used for plotting of graphs and data representation. The results were expressed as Means \pm Standard deviation for triplicate determinations ($n = 3$) and compared at 0.05 level of significance.

Results and Discussion

Solid-state fermentation technique is a vital microbial process that is carried out in conditions that resemble the natural habitat of the employed microorganism (especially a fungus) in a medium with little or no free water. In this microbial technique, the solid substrate serves as the housing medium providing all necessary conditions required for the microbial cell growth (Sadh et al., 2018; Anigboro et al., 2020b). This fermentation protocol has been shown to enhance the nutritional qualities of foods such as increased bioavailability, biosafety and levels of essential nutrients (Oyarekua, 2013; Morgan and Choct, 2016; Aganbi et al., 2020; Anigboro et al., 2020a).

various biochemical parameters were evaluated in all the different-inoculum-size fermented samples as well as in the control (that is, the unfermented sample). From the results obtained (Figure 1), the pH of all

inoculated samples was reduced while that of the control (0% inoculum-size-fermented sample) remained unchanged. The 5, 20, 25 and 30% inoculum-size-fermented samples were observed to be more acidic than the 10 and 15% ones. Oyarekua (2013) reported a similar decrease in pH of co-fermentation process of maize (50%), cowpea (30%) and sweet potato

(20%w/w) for the production of complementary infants' food. Organic acids are reportedly produced by the metabolic activities of fungi during fermentation (Oduah et al., 2015). However, the observed pH reduction in the fermented medium in this study may be due to increased amounts of organic acids produced by the metabolic activities of the fungi.

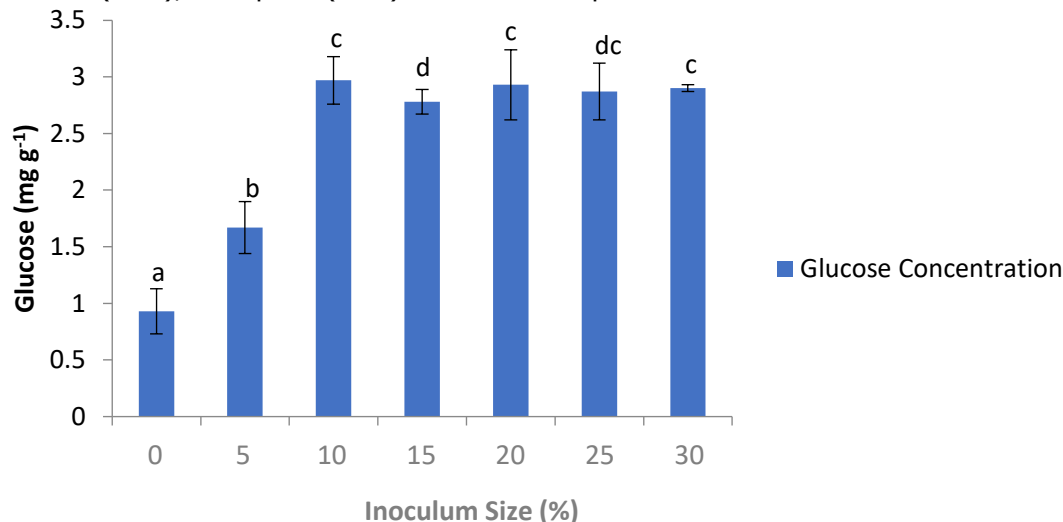


Figure 2: Glucose levels of different-inoculum-size fermented *C. esculenta* using *R. oligosporus*. Different letters differ substantially at $p < 0.05$ on each bar, which is a representation of mean SD and represents triple observations ($n=3$).

The levels of glucose and proteins were increased statistically ($p < 0.05$) in all the fermented samples (with the highest values observed at 2.97 ± 0.21 and 29.8 ± 1.01 mg/g respectively at 10%) when compared to the unfermented samples (0.93 ± 0.20 and 8.48 ± 0.36 mg/g respectively at 0%) (Figures 2 and 3). According to Oboh and Akindahunsi (2003), there is breakdown of complex polysaccharides into simpler carbon sources required for several metabolic purposes. Also, increased

protein level was attributed to the microbial secretion of extracellular proteins and enzymes for their metabolic processes during the fermentation process (Oseni and Ekperigin, 2007; Egbune et al., 2021). The increase in glucose and proteins observed in this study is in consonance with the findings of Anigboro et al. (2020a) during the fermentation of maize (*Zea mays*) offal using the same fungus employed in this study.

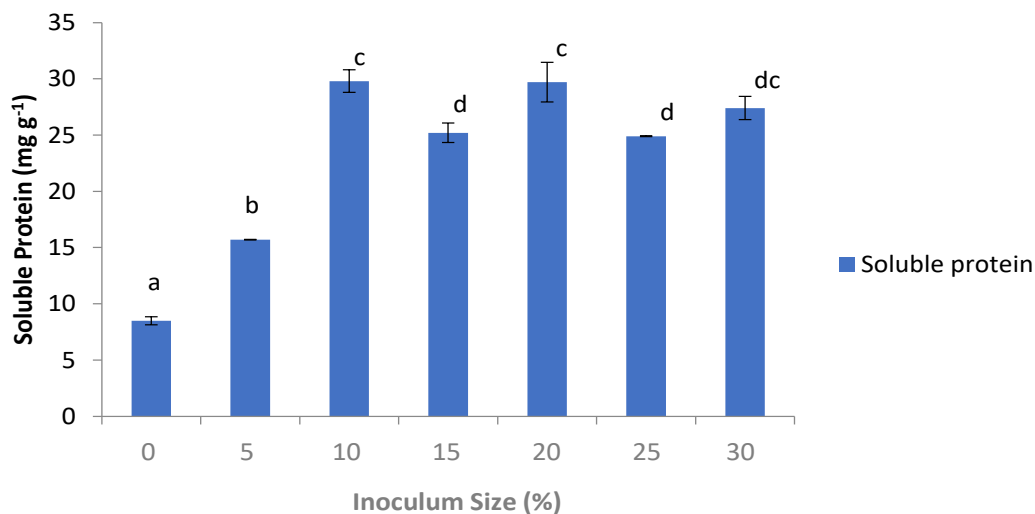


Figure 3: Protein levels of different-inoculum-size fermented *C. esculenta* using *R. oligosporus*. Different letters differ substantially at $p < 0.05$ on each bar, which is a representation of mean SD and represents triple observations ($n=3$).

In contrast to the observation with glucose and soluble proteins levels, the result of this study (Figure 4) showed significant decrease in the levels of reducing sugars in all the fermented *C. esculenta* samples (with lowest value of 1.74 mg/g observed in the 20 and 25% inoculum-size-fermented samples) compared to the control (3.44 ± 0.06 mg/g). Earlier studies have established an increased susceptibility of starch to

hydrolysis with increased levels of reducing sugars (Anigboro et al., 2018; Coronell-Tovar et al., 2019; Ojo et al., 2022). The reducing sugars levels in the samples declined with increasing inoculums size except at 30% inoculum size. This suggests that consumption of the fermented cocoyam will be beneficial to diabetic patients (Awa and Eleazu, 2015).

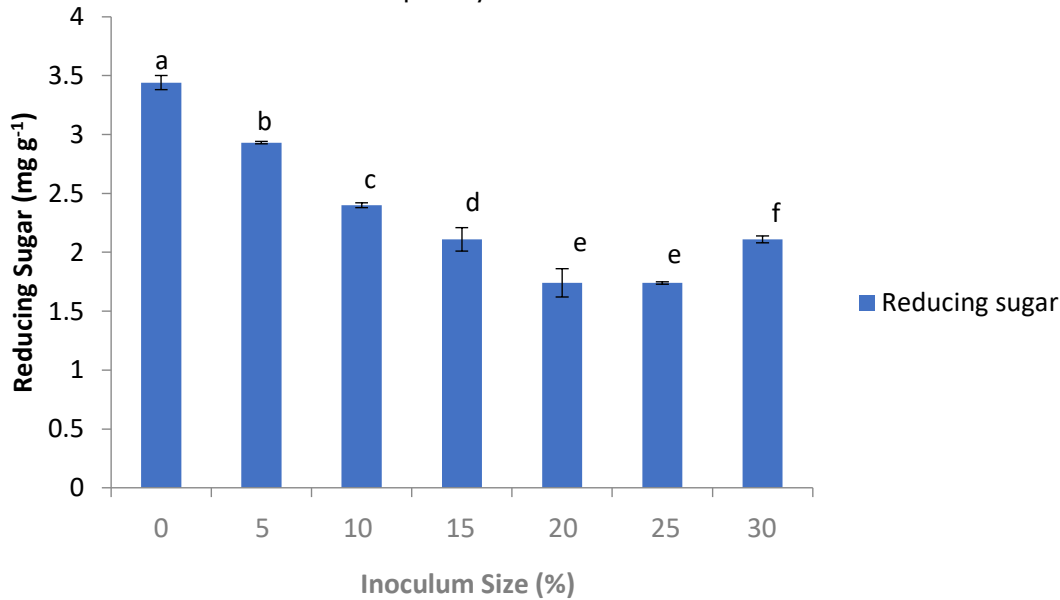


Figure 4: Reducing sugar concentrations of different-inoculum-size fermented *C. esculenta* using *R. oligosporus*. Different letters differ substantially at $p < 0.05$ on each bar, which is a representation of mean SD and represents triple observations ($n=3$).

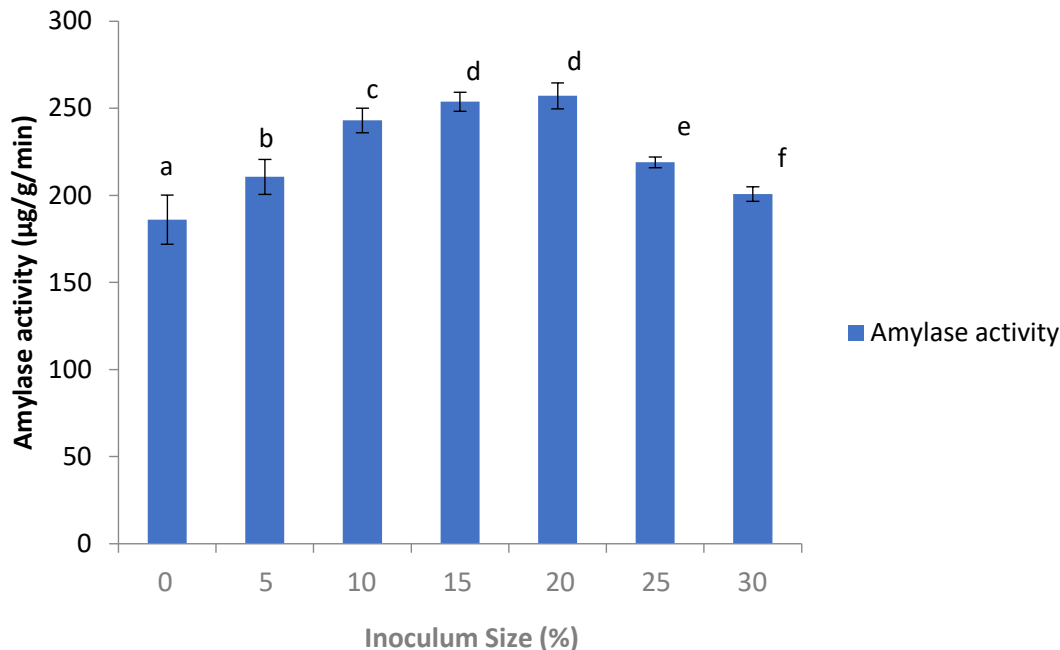


Figure 5: Alpha-amylase activity of different-inoculum-size fermented *C. esculenta* using *R. oligosporus*. Different letters differ substantially at $p < 0.05$ on each bar, which is a representation of mean SD and represents triple observations ($n=3$).

The result of α -amylase activity (Figure 5) revealed significant increase ($p < 0.05$) in all inoculum sizes, with the sample fermented at 20% inoculum size shown to have the highest amylase activity. This increase in amylase activity may be attributed to the fungal release of extracellular carbohydrates digesting enzyme for the hydrolysis of starch into simple sugars required for microbial metabolism. Most saprophytic fungi growing on organic food substances are known to derive nutrients materials from such complex foods by secreting digestive exogenous enzymes for their catabolism to simpler biomolecules. Generally, α -amylases catalyze the hydrolytic cleavage of O-glycosidic linkages in starch or cellulose molecules into disaccharides and monosaccharides for several metabolic uses (Nouadri et al., 2010; Metin et al., 2010; Egbune et al., 2022). This could be the possible rationale behind the increase observed in this study.

Conclusion

The results of this study indicated that the solid-state fermentation of cocoyam with *R. oligosporus* significantly enhanced its nutritive value (proteins, glucose and α -amylase). The *R. oligosporus* fermented cocoyam could find application in human and animal feed production industries, and/or serve as a good substrate for industrial production of important biomolecules.

Conflict of Interests

The authors have not declared any conflict of interests.

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