CHANGES IN GLUCOSE, AMYLASE AND SOLUBLE PROTEINS LEVELS IN SOLID STATE FERMENTED YAM (*DIOSCOREA* SPP.) PEELS BY *RHIZOPUS OLIGOSPORUS*

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Solid-state fermentation (SSF) of yam peels was carried out by *Rhizopus oligosporus* under acidic and basic conditions, to optimize the production of soluble proteins, glucose and α-amylase activity. Cultures were prepared with dried, grounded yam peels as substrate . The substrate was inoculated with spores of *R. oligosporus* after pH adjustment using citrate and phosphate buffer solutions. Five sets of samples with pH range of 3 – 7 (citrate cultures), and 7 – 11 (phosphate cultures) were obtained. Each sample set had a control at pH 6; all samples were left at room temperature and allowed to ferment for four days. Post-fermentation, there was noticeable decrease in acidity in the citrate cultures and increase in acidity for the phosphate cultures; this in turn had an effect on α-amylase activity and the production of glucose and total soluble proteins. Decreased acidity resulted in increased production of soluble proteins and amylase activity (247.47 ± 3.00 mg g^{-1} and 420.09 ± 21.45 μg g^{-1} min^{-1} respectively), compared to the control (118.13 ±13.30 mg g^{-1} and 312.42 ± 14.04 μg g^{-1} min^{-1} respectively). Changes observed in both parameters were significant ($p < 0.05$); by contrast, glucose level reduced with rise in pH by 4.7 units but peaked after a rise by 1.8 units. The best initial pH for optimum amylase and soluble proteins production using citrate as buffer is 3, while that for glucose production is 6. With phosphate buffer, the highest glucose content was obtained in the sample at initial pH 11 after a decrease in pH by 3.1 units (increase in acidity to pH 7.9). The highest yield for soluble proteins (222.13 ± 20.41 mg g^{-1}) and amylase (377.96 ± 21.45 μg $g^{-1}min^{-1}$)was observed at initial pH 8; this increase was significant compared to control ($p < 0.05$). Overall, for both buffer solutions, increases in glucose yield was not significant compared to control ($p > 0.05$). However, the results confirm that *R. oligosporus* could be used for amylase and soluble proteins production by SSF on yam peels. Citrate buffer is recommended as the most suitable for pH control using yam peels as solid medium material.

Key words: Solid state fermentation, yam peels, *Rhizopus oligosporus*, α-amylase activity.

INTRODUCTION

The need to produce value added products from agricultural wastes like yam peels as a means to reducing environmental pollution is now a major issue of concern. A recurring problem for developing countries is improper management of wastes generated from anthropogenic activities; in particular agricultural wastes. More challenging is the unsafe disposal of these wastes into environments like fresh water reservoirs which become unsuitable for inhabiting species (Fakayode, 2005). Yam (*Dioscorea* spp.) is a multi - species, polyploid and vegetatively propagated tuber crop that is cultivated widely in the tropics and sub-tropics (Mignuona et al., 2003). Yams are economically important as a staple food source of carbohydrates among other nutrients; they are also known to be rich in polyphenolic compounds like catechins, anthocyanins etc. which have immense medicinal benefits (Farombi, 2003; Bassey, 2017). Furthermore, they have immense cultural value since it is the only crop celebrated in Nigeria during and after harvest in the form of a *Yam Festival*. Previous records have indicated a steady increase in yam production in Nigeria, with the country implicated as the largest world producer

of yams at an annual production amount of at least 45 million metric tonnes (Bassey, 2017). This makes Nigeria a major producer among countries described as 'Africa's Yam Belt'. Consequently, large amounts of yam peels result from the high production and processing rates of yams for various purposes in Nigeria.

Yam peels are often discarded as waste, even though studies have indicated that the peels may be useful as ingredients for animal feeds. For example, Ekeyem et al. (2006) described the addition of 15% grounded yam peels in a maize diet formulation that was successfully fed to broiler chicken without having adverse effects on their growth or overall performance. Yam peels have been reported as low in nutrients like proteins and vitamins, yet known to be rich in fiber and starch; which are often poorly digestible by livestock animals (Akinfemi et al., 2009; Ogbuewu et al., 2010). Recent FAO reports on world food production indicate faster growth rate for livestock and biofuel production over crop production (FAO, 2018). Presumably, the current trend of shift in the production of cereal foods like wheat and rice towards coarse grains and oilseeds to meet demand for animal feed and biofuel production further impresses on the need for alternative sources of raw materials for the processing and formulation of animal feeds.

The use of microbial fermentation to enhance the nutritional quality and digestibility of various agricultural wastes has long been exploited. These processes often lead to the production of value-added products like enzymes, proteins, citric acid, etc. Solid-state fermentation (SSF) has shown much promise as a sustainable approach for the bioconversion of solid wastes due to its relative simplicity, high efficiency in terms of products yield, low water and energy consumption among others, with the added advantage of safe disposal of agro-industrial wastes (Soccol et al., 2017; Sadh et al., 2018; Benabda et al., 2019). Several bacteria and fungi species, including *Aspergillus niger*, *Saccharomyces cerevisae* have been widely utilized as starter culture during SSF for the production of enzymes and other value-added products (Sadh et al., 2018).

Rhizopus oligosporus has been less commonly utilized; this filamentous fungus is a GRAS (Generally Regarded as Safe) organism that grows rapidly at 34-45°C. It assumes various morphological hyphal formation and has been successfully used in the SSF of *tempe* (a traditional fermented Indonesian food, produced from soybean) (Miszkiewicz et al., 2004). This study explored the suitability of *R. oligosporus* as starter culture for the conversion of yam peels into a more nutritive component with potential for use as animal feedstock. Specifically, the effect of pH on α-amylase activity and the concentrations of glucose and soluble proteins produced during SSF by *R. oligosporus* were determined. Hitherto, no data on solid state fermentation of yam peels by *R. oligosporus* have been reported.

MATERIALS AND METHODS

Collection and preparation of plant material

Yam (*Dioscorea* spp.) peels discarded as waste were collected in Abraka, Delta State in the month of December 2018 and air dried for about three weeks. Dried peels were grinded and stored at room temperature until required for analysis.

Preparation of starter culture – The inoculum

R. oligosporus cells (manufactured by PT Aneka Fermentasi Industri, Bandung-Indonesia) were obtained from Harmony Path. Ltd. laboratory located at Songhai in Amukpe, Sapele Delta State. Cells were resuscitated by sub-culturing onto potato dextrose agar (PDA) plate prepared according to the manufacturer's instruction. The plates were incubated at 30°C for7 days for complete sporulation. Following this, the spores were scraped with an inoculating loop under aseptic conditions to prepare spore suspension that was used as the inoculum for the fermentation.

Solid state fermentation and preparation of extracts

Seven grams of grounded yam peels was placed in five Petri dishes and served as substrate. Spores of *R. oligosporus* weighing approximately 1 g were homogenized separately, in 15 ml of citrate and phosphate buffer solutions adjusted to five different pH levels (citrate = pH 3- 7; phosphate = pH 7- 11). Homogenized cell suspension was added to Petri dishes containing substrate. Content was mixed thoroughly and labeled

according to the corresponding pH. The Petri dishes were then covered and the mixture was allowed to ferment for 4 days at room temperature; pH was determined using a pH meter at the end of the fermentation period. An unfermented control (containing dried and grounded peels, devoid of any presence of molds; with buffer only, and without any cells) was prepared alongside the test samples. At the end of the fermentation period, 3.1 g of the mixture was taken from each of the Petri dishes; 40 ml of distilled water was added and homogenized using a mortar and pestle. 10 ml of homogenized mixture was collected in a test tube and then centrifuged for 10min. The supernatant was collected in sterile universal containers and used as the crude extract or sample for the various analytical assays.

Analytical procedures

The following analytical procedures were carried out in triplicates using the crude extract obtained from above. Total soluble proteins were determined according to the method described by Gornall et al. (1949). Protein standard (Bovine albumin) solutions in the concentration range of 0.5- 10 $mgml^{-1}$, 0.5 ml aliquots were placed in test tubes; 0.5 ml of each of the test samples was also placed in series of test tubes. 2.5 ml of Biuret reagent was added to each test tube containing test sample or standard; it was vortexed and allowed to react for 30 mins. Distilled water was used as blank. After the incubation time, the absorbance of standards and test samples was read at 540 nm.

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The procedure as described by the Randox kit (Randox Laboratories Ltd, UK) was followed. Briefly, 20 µl of standard and the various test samples were pipetted into test tubes labeled in triplicates. 2 ml of the reconstituted glucose working reagent was added to each test tube. Distilled water was used as blank. The content of the test tubes was mixed and incubated for 25 min at 25°C. After the incubation time, the absorbance of standard and test samples was read at 500nm. Glucose concentration ($mgdl^{-1}$) was obtained from the equation:

$$Glucose\ Conc. = \frac{Abs\ sample}{Abs\ standard} \times Standard\ conc. that\ is\ 1.02\ mg/ml$$

Alpha-amylase activity was determined according to the method described by Nouadri et al. (2010) with some modifications. 0.5 ml of 1% (w/v) soluble starch in 0.1 M phosphate buffer (pH 6.5) and 0.5 ml of test sample were pipetted into labeled test tubes. Also, 0.2% (w/v) standard maltose solution in the range of 0 - 2 $mgml^{-1}$was prepared to a final volume of 1 ml. A test tube containing blank solution was also prepared. All test tubes were incubated at 30°C for 30 min. After the incubation, the reaction was stopped by addition of 2 ml of 3,5-dinitrosalicylic acid (DNS) reagent. Test tubes were placed in boiling water for 5 mins for colour development followed by cooling. 10ml of distilled water was added to all test tubes prior to reading absorbance at 540 nm. A standard calibration curve was plotted and used to determine the activity of amylase in test samples. One unit (U) of amylase activity was defined as the amount of enzyme that released 1 µg of maltose (as reducing sugar equivalent) per ml of sample per min under the assay conditions.

$$Enzyme\ activity\ = \frac{\mu g\ of\ maltose\ released}{Volume\ (in\ ml) of\ enzyme\ used\ \times\ incubation\ time\ (30\ mins)}$$

Statistical analysis

All data were subjected to statistical analysis. Values were reported as Mean ± Standard deviation and the experimental results were analyzed using analysis of variance (ANOVA) and also a Fischer test of least significance (LSD) was carried out to compare the various group means. The results were considered significant at p-values of less than 0.05, that is at 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

Solid state fermentation of yam peels, a readily abundant agro waste product, by *R. oligosporus* under acidic and basic conditions was carried out for four days to optimize the production of

soluble proteins, glucose and α-amylase activity. For cultures with citrate buffer solutions, there was a decrease in acidity as observed in increase in measured pH values post-fermentation, while for phosphate buffer cultures, there was increase in acidity as seen from a drop in pH values measured after the fermentation process (Table 1).

Table 1. Changes in pH before and after fermentation according to buffer solution used.

Sample	Control	A	B	C	D	E
Citrate buffer						
pH before fermentation	6.0	3.0	4.0	5.0	6.0	7.0
pH after fermentation	6.0	7.7	7.8	7.9	7.8	8.0
Change in pH	0.0	4.7	3.8	2.9	1.8	1.0
Phosphate buffer						
pH before fermentation	6.0	7.0	8.0	9.0	10.0	11.0
pH after fermentation	6.0	7.8	7.8	7.9	7.8	7.9
Change in pH	0.0	0.8	0.2	1.1	2.2	3.1

Effect of decreasing acidity on measured biochemical parameters

As shown in Table 1, citrate buffer solutions in the pH range of 3.0- 7.0 before fermentation became less acidic as fermentation ensued. The effect of this decrease in acidity on the levels of glucose, total soluble proteins and amylase activity is illustrated in Figure 1.

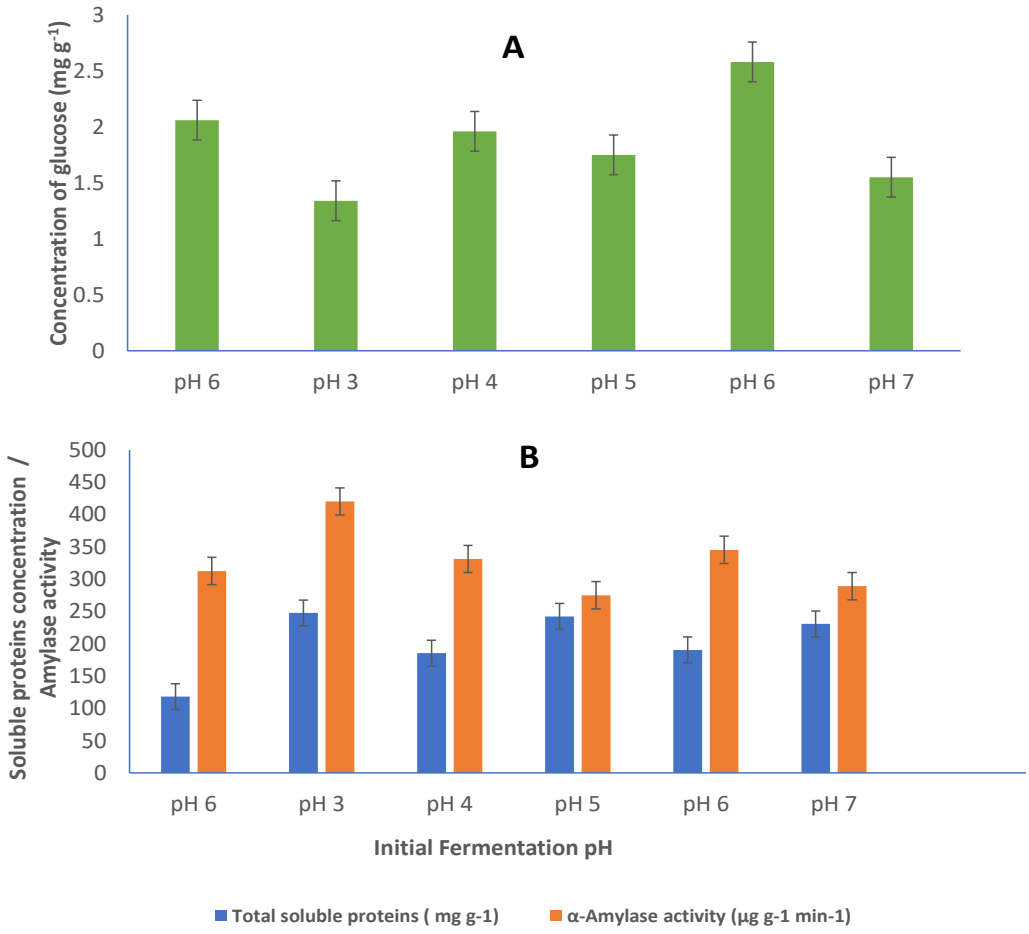

Figure 1. Change in the levels of glucose (A), total soluble proteins and α-amylase activity (B) with decreasing acidity (increasing pH) during SSF by *R. oligosporus*. pH 6 = control, pH 3, 4, 5, 6 and 7 = test samples.

For total soluble proteins and α-amylase activity, there was a change in concentration following a rise in pH by 4.7 units (that is, sample A, with an increase in pH from 3 to 7.7) after fermentation (Table 1). This decrease in acidity resulted in increased production of soluble proteins as well as increased amylase activity (247.47 ± 3.00 mg $g^{-1}$and 420.09 ± 21.45 μg g^{-1} min^{-1}respectively). Compared with the control (118.13 ±13.30 mg g^{-1} and 312.42 ± 14.04 μg g^{-1} min^{-1} respectively) which was at pH 6 before and after fermentation, the change in total soluble proteins concentration and amylase activity was significant ($p < 0.05$). For soluble proteins, subsequent decrease in acidity resulted in a non-uniform decrease in concentration; amylase activity also reduced with the rise in pH. The implication for both parameters during solid state fermentation of yam peels by *R. oligosporus* using citrate as buffer is that the best initial pH for medium preparation is 3; and as fermentation goes underway, there is a gradual increase to an optimum pH of 7.7 (equivalent to a rise in pH by 4.7 units) where the highest production of soluble proteins and amylase activity was observed. In contrast, glucose concentration reduced (1.34 ± 0.78 mg g^{-1}) with decreasing acidity and rise in pH (by 4.7 units from pH 3 to 7.7); the decrease was not significant when compared with the unfermented control (2.06 ± 0.47 mg g^{-1}). Further decrease in acidity resulted in a peak in glucose concentration at pH 6, equivalent to a rise in pH by 1.8 units to 7.8 (2.58 ± 0.89). Though, this change was not significant ($p > 0.05$). Accordingly, the best initial pH for optimum glucose production during SSF of yam peels using citrate as buffer, and *R. oligosporus* as starter culture is 6.

Effect of increasing acidity on measured biochemical parameters

Using phosphate buffer solution for pH control, the initial pH in culture plates were: Control - pH 6, samples A – pH 7, B – pH 8, C – pH 9, D – pH 10 and E - pH 11. With the exception of Sample A, where final post-fermentation pH increased slightly by 0.8 units (from 7 to 7.8), there was observed decrease in final pH by several units ranging from 0.2- 3.1 in all other samples. The final pH and change in pH for the samples with phosphate buffer solution are shown in Table 1. In general, for the test samples the trend was increasing acidity (with decrease in pH) when phosphate buffer was used for pH control. The implication for glucose, soluble proteins and α-amylase production is illustrated graphically in Figure 2 and discussed next. Glucose concentration was lowest at the initial pH of 7 (1.03 ± 0.47 mg g^{-1}, corresponding to post-fermentation pH of 7.8) and the decrease was significant compared to that of unfermented control ($p < 0.05$); the highest glucose content was noticeable at initial pH 11 following a decrease in pH by 3.1 units to 7.9 (2.47±0.31 mg g^{-1}). This increase in glucose concentration was not significant compared to the control ($p > 0.05$). The highest yield for soluble proteins was observed at initial pH 8 (222.13±20.41 mg g^{-1}, corresponding to post-fermentation pH of 7.8). This increase was significant compared with unfermented control ($p < 0.05$). Alpha-amylase activity was also the highest at initial pH 8 (377.96±21.45μg $g^{-1}min^{-1}$) following a decrease in pH to 7.8. This increase was significant compared to control ($p < 0.05$). Overall, the use of phosphate buffer in the pH range of 7- 11 for fungal growth resulted in increasing acidity post-fermentation due to decrease in pH values to an optimum pH of 7.8 where the yield for soluble proteins and α-amylase was highest. For this choice of buffer solution, the best initial pH for culture preparation during SSF on yam peels by *R. oligosporus* for proteins production is 8 while that for optimum glucose yield is pH 11.

Reports from various studies have recommended optimization of factors like: pH of medium, fermentation time, temperature, moisture content, inoculum size, choice of buffer solution etc. during SSF. This is because these factors directly affect microbial growth and hence yield of desired products (Benabda *et al*., 2019). The effect of the use of different nitrogen sources and buffer solutions on the growth of *R. oligosporus* during SSF of apple pomace for protein production has been reported. Phosphate buffer was found to be the most suitable for pH control while urea was a better nitrogen source favoring high protein yield (Albuquerque et al., 2006). The use of *Rhizopus oryzae* and other *Rhizopus* species in SSF for the production of

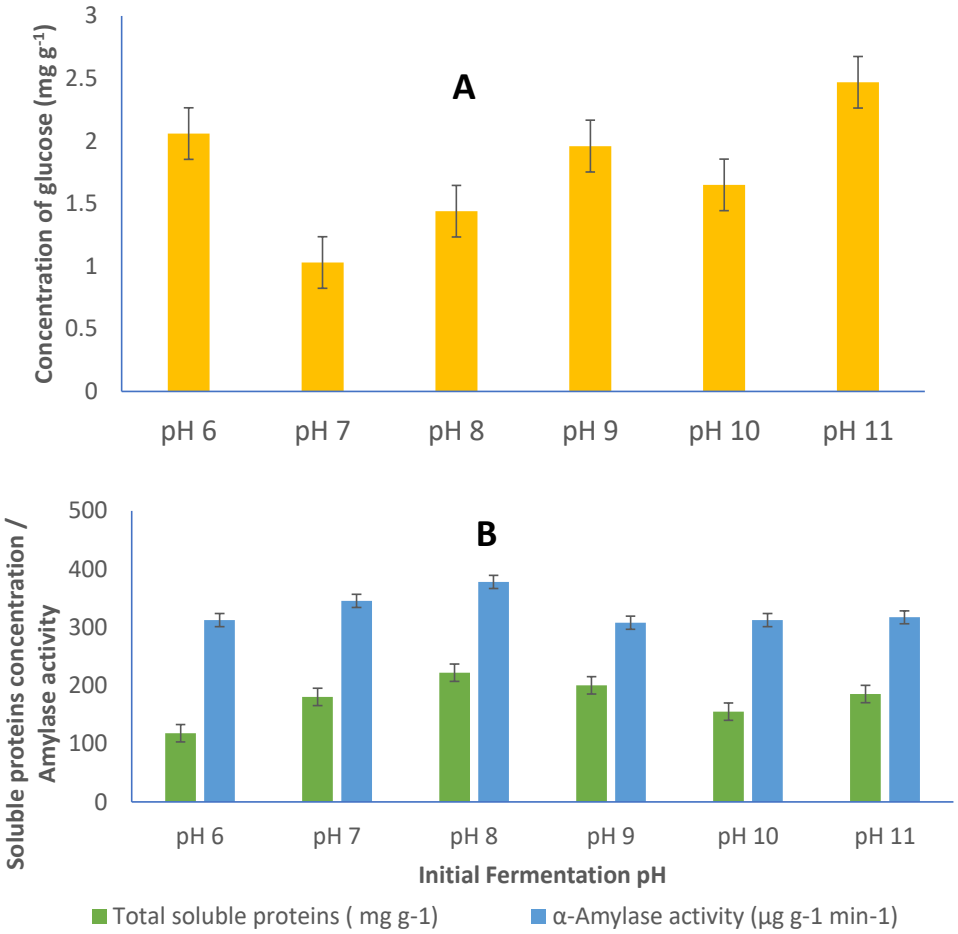

Figure 2. Change in the levels of glucose (A), total soluble proteins and α-amylase activity (B) with increasing acidity (decreasing pH) during SSF by *R. oligosporus*. pH 6 = control, pH 7, 8, 9, 10 and 11 = test samples.

value-added products from food crops and agro-industrial wastes has been a long-standing practice. However, the utilization of *R. oligosporus*as suitable starter organism in such fermentation processes has been least reported. This study demonstrated the use of *R. oligosporus* in the solid-state fermentation of yam peels, using citrate and phosphate buffer solutions in separate media preparations for pH control and recorded increase in α-amylase activity, as well as in the levels of glucose and total soluble proteins. Previous workers have demonstrated the use of *R. oligosporus* in SSF of some agricultural wastes for the enhancement of protein and minerals contents among other nutrients (Belewu and Babalola, 2009). While similar improvement in protein content was recorded in this study, most of the available studies have focused on a range of agricultural wastes other than yam peels. The successful utilization of yam peels as a new raw material for SSF by *R. oligosporus* with the view to obtaining fermented products with improved nutrients, for use either wholly or as part of formulations of animal feedstock is innovative. However, there is need to extend work to include studies to evaluate the effect of the fungus on the composition of micronutrients, the activity of other hydrolytic enzymes, and how these in turn affect aesthetic values of the fermented yam peels. Also, the overall efficiency of *R. oligosporus* fermentation in the removal of anti-nutritive components that may be present in fermented yam peels need to be explored further.

Nevertheless, by the outcomes reported in this study, *R. oligosporus* increases the range of microorganisms, in particular, the fungal species that could be used for amylase, glucose and proteins production by SSF with yam peels as an ideal substrate. Based on the yield obtained in this study, the citrate buffer is recommended as the most suitable for pH control using yam peels as

solid medium material.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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