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CHANGES IN BIOCHEMICAL PARAMETERS DURING THE SOLID-STATE FERMENTATION OF PINEAPPLE (*Ananas comosus* L. Merr) PEELS BY *Rhizopus oligosporus*

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ABSTRACT

Enzymes production and other relevant biomolecules from environmental wastes could be easily enhanced or hindered by slight alterations in the bioprocess. In this study, the impact of pH variations on amylase production by Rhizopus oligosporus during the solid state fermentation of pineapple (Ananas comosus L. Merr) peels was investigated. After five days of fermentation, the variations in glucose and soluble protein levels of the fermented pineapple peels were also investigated. The results established a notable increase (p<0.05) in the levels of soluble proteins and glucose in the fermented pineapple peels at all the pH values tested (pH 3 to 9); however, while they were highest at pH 3, amylase activity was highest at pH 6. This preliminary study shows that solid state fermented pineapple peels are rich in glucose, soluble proteins and amylase.

Keywords: Amylase, fermentation, pineapple, Ananas comosus, Rhizopus oligosporus

INTRODUCTION

Pineapple peels are waste products from industrial pineapple juice production. They are dumped indiscriminately in the environment and may cause hazards such as greenhouse effect. They are even harmful to human and animal health since they are neither treated biologically nor chemically (Bos and Hamelinck, 2014). These industrial or domestic wastes are posing serious disposal challenges (Rodríguez-Couto, 2008; Okonko et al., 2009); however, they contain a lot of proteins, sugars and minerals which can be transformed to usable forms (Graminha et al., 2008). Under nutrition has long been a challenge due to a major drop in foodstuff intake, and there is a need to considerably boost food intake, particularly protein, through the use of unconventional protein bases such as protein-enhanced agricultural and food processing decay (Anigboro et al., 2014; Aruna 2019).

Biotransformation of agricultural residues using proper technical techniques can remove waste pollutants, reduce the amount of trash generated yearly, reduce waste disposal concerns, and offer economically useful products with adequate protein quantity and quality (Ojo et al., 2022; Ndego et al., 2022). This is conceivable because these wastes still include nutrients (most notably carbohydrates, minerals, Published articles have clearly explained the use of different waste materials like organic manure, soil dust, cassava and pineapple peels for and proteins) that can aid microbes to thrive on (Aworh, 2010).

Because pineapple peels have sugars that can promote the development of microbes like *Saccharomyces cerevisiae, Candida utilis*, and *Trichoderma viride*, turning these agricultural wastes into economical, beneficial, and safe items will be a successful business venture (Meena et al., 2021; Egbune et al., 2021a). Furthermore, as the demand for new protein sources grows on a daily basis, it is vital to optimize the consumption of lignocellulosic residues, such as pineapple peels, in the production of safe and highly nutritious animal feeds, reducing reliance on traditional food protein sources (Aganbi et al., 2020; Awasthi et al., 2021).

Earlier studies focused on the influence of exogenous nitrogen sources such as urea, nitrate, and ammonium sulphate on biomass and protein synthesis during fermentation since fermenting bacteria often prefer one source over another. Duru and Uma (2003), for example, found that Aspergillus oryzae used urea more efficiently than alternative nitrogen sources during protein enrichment of cocoyam solid wastes. Furthermore, Awoyele (2010) found that *T. viride* and ammonium sulphate produced more protein and biomass than urea during solid state fermentation (SSF) of cassava peels. alcohol, enzymes, natural antimicrobials and animal feed production (Anigboro, 2012a, b; Tonukari et al., 2015; Avwioroko et al., 2016;

Anigboro et al., 2020). Several microorganisms possess the capability to biotransform agricultural wastes to useful products through SSF processes. However, no data on SSF of pineapple peels by the fungus *Rhizopus oligosporus* has been reported. This research aims to explore pineapple peels as source of soluble proteins, glucose and amylase production by *Rhizopus oligosporus* using solid state fermentation at different pH levels.

MATERIALS AND METHODS

Materials

Peels of pineapple (*Ananas comosus*) were amassed at Abraka Community, Delta State. They were identified and given voucher number (UBH- A234) in the Department of Botany, University of Benin, Edo State, Nigeria. After seven days of air-drying, the peels were grinded into powder form and kept at 37^{o}C.

Solid state fermentation technique

Solid state fermentation was estimated as described by (Egbune et al., 2022;Ofuya and Nwanjuiba, 1990). One gram of *R. oligosporus* (1.4 × 10^2 CFU) was added separately to 15 ml of citrate and phosphate buffers, mixed properly and added to seven pH (pH 3 to 9) labeled different Petri dishes. To each of the Petri dish, 7 g of the powdered peels was added, mixed, covered and left to undergo fermentation for a period of five days. 40 ml of distilled water was added to 3.1 g of the collected fermented mixture and homogenized with the help of mortar and pestle. 10 ml of the homogenized mixture was taken and centrifuged for 10 min. The supernatant (crude sample) was collected into universal containers and stored at 4°C for the different assays.

Estimation of biochemical parameters

pH was measured by Extech pH meter. Glucose and total soluble protein were determined in line with Randox glucose kit protocols following strictly manufacturer's specification (Trider, 1959) and Biuret method (Gornal et al., 1949), respectively. Lever (1972) method was adopted for amylase activity and its activity was expressed as µg of maltose released by the enzyme per gram of the sample per minute.

Statistical analysis

Analysis of variance (ANOVA) was used. Values were reported as mean ± standard deviation. The results were assumed significant at p-values of less than 0.05(95%) confidence level ($p<0.05$).

RESULTS

Effects of solid state fermentation on the pH of pineapple peels

The pineapple peels were fermented at different pH levels (pH 3 to 9). The final pH values after solid state fermentation for 5 days are shown in Figure 1. While the unfermented control sample has a pH of 4.6, all the pineapple peels fermented at pH 3 to 8 have a final pH of 5.5 to 6.5. The pineapple peel samples fermented at pH 9 have a final pH of 7.5 after 5 days of fermentation. The result showed that (except for the sample fermented at pH 9), irrespective of the starting pH, *R. oligosporus* is able to ferment the pineapple peels, modulating their pH to slightly acidic range of 5.5 to 6.5.

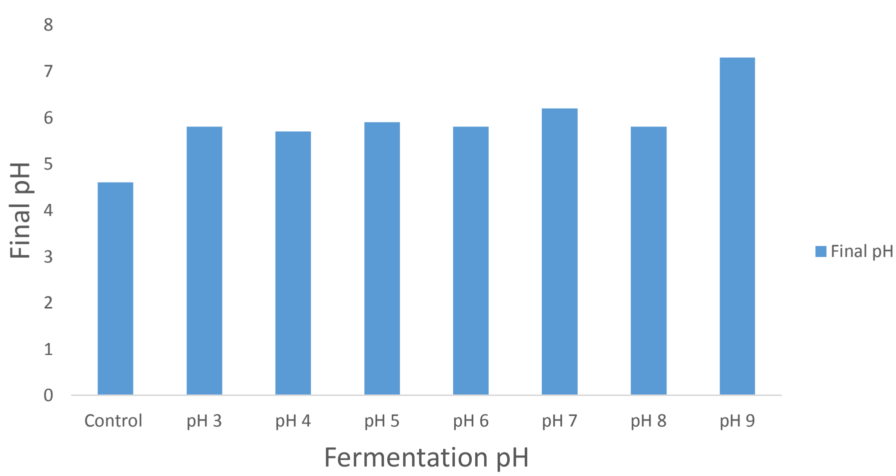

Figure 1. Final pH of *R. oligosporus* fermented pineapple peels (at pH 3 to 9) after 5 days.

Effect of solid state fermentation on soluble proteins

The results of total soluble proteins revealed that soluble proteins levels significantly increased ($p<0.05$) at all the pH levels (pH 3 to 9) investigated compared to the control (Figure 2). The increasing order of the soluble proteins concentration released at the different fermentation pH values is pH 8 > 5 > 6 > 7 > 9 > 4 > 3.

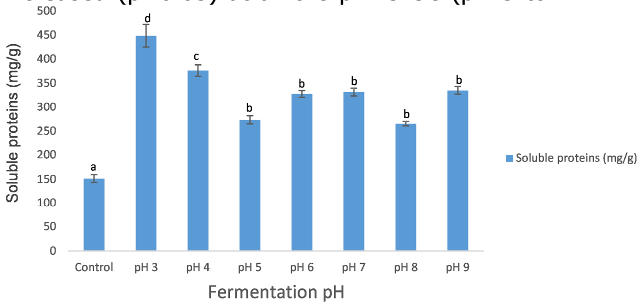

Figure 2. Soluble proteins concentration during SSF of pineapple peels by *R. oligosporus* at different pH. Relative to the control, bars with different attached alphabets are statistically different (that is, $p<0.05$).

Effect of pH on glucose levels

Glucose levels were determined at different fermentation pH levels (pH 3 to 9) after five days of fermentation (Figure 3). The results demonstrated a general marked increase in glucose levels at all the pH levels tested but highest at pH 3 in relation to the control ($p<0.05$).

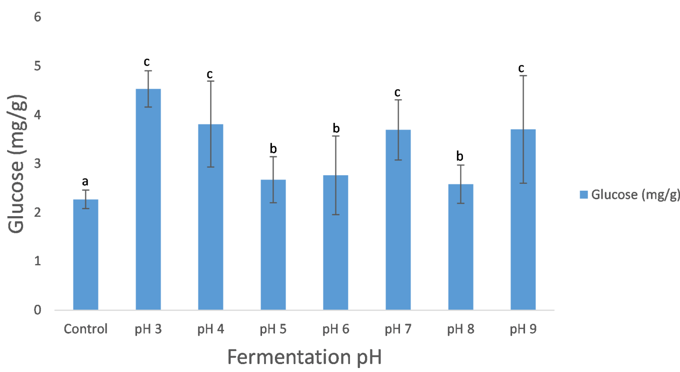

Figure 3. Glucose levels during SSF of pineapple peels by *R. oligosporus* at different pH. Relative to the control, bars with different attached alphabets are statistically different (that is, $p<0.05$).

Effect of pH on amylase activity

The results of amylase activity after fermentation for five days expressed as µg of maltose released by the enzyme per gram of the sample per minute are presented in Figure 4. Amylase activity momentously increased at pH 5 to 9 compared to the control ($p<0.05$). However, the highest amylase activity was observed at pH 6.

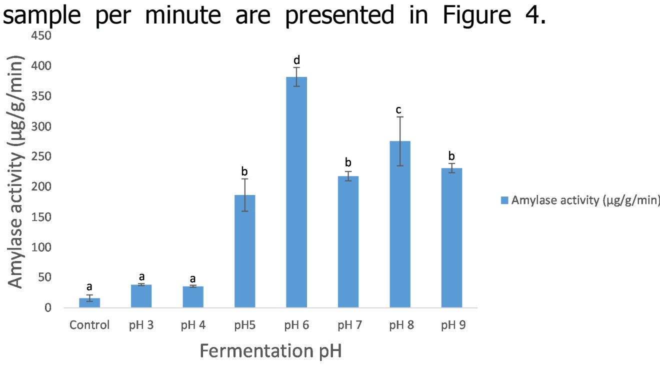

Figure 4. Amylase activity during SSF of pineapple peels by *R. oligosporus* at different pH. Relative to the control, bars with different attached alphabets are statistically different (that is, $p<0.05$).

DISCUSSION

Solid state fermentation has a wide range of industrial application, with wonderful qualities. It is a great way of converting different types of agro wastes into useful compounds or improving the proteins, carbohydrates and other components of these wastes (Noraziah et al., 2017; Egbune et al., 2022; Anigboro et al., 2022). Fungi have the capability to secrete an array of enzymes of which *R. oligosporus* has been shown to be a suitable source of amylase (Hamlyn, 1998). This enzyme breaks down the complex carbohydrates in the substrate into soluble sugar. Traditional or spontaneous fermentation, a process of removing acids in fruits and vegetables has been in existence for centuries (Casado et al., 2017). Unfermented pineapple peels as seen in this work have pH value of 4.6 (Figure 1) which was slightly brought to acidic range of 5.5 to 6.5 after fermentation by *R. oligosporus*. The reduction in acidity observed in the medium suggests that *R. oligosporus* can remove excess acids from the medium. This report harmonizes with the previous work of Casado et al. (2017) and Susilowati et al. (2018). Soluble proteins are very crucial to humans since they are required for everyday activity. The conventional sources of protein are inadequate to meet up the demand of our increasing population. Insects and microorganisms have been harnessed as other sources of proteins for humans. Agricultural wastes are not left out; many have been bioconverted and utilized in animal feed formulation (Avwioroko et al., 2016; Anigboro et al., 2020).

pH improved the concentration of soluble proteins during solid state fermentation at all the pH levels studied but marked increase of 448.67 ± 23.33 mg/g of soluble proteins was observed at pH 3 in relation to the control (Figure 2). This suggests that the solid state fermentation of pineapple peels at pH 3 (strong acidity) favoured the release of proteolytic enzymes by the fungus, on one hand or modulated the bromelain activity present in the pineapple peels which catalyzed the degradation of proteins, on the other hand. This report validates the previous work of Sarkar et al. (2018). Bioconversion of pineapple waste by *Saccharomyces cerevisiae* increased protein content (Roberta et al., 2007).

Glucose concentration during solid state fermentation of pineapple peels by *R. oligosporus* increased remarkably ($p<0.05$) at all the pH values investigated when compared with the control (Figure 3). The highest glucose level (4.53 ± 0.37 mg/g) was seen at pH 3 indicating strong acidity. This point out that solid state fermentation of pineapple peels by *R. oligosporus* increased the levels of glucose and may be due to the degradation of polysaccharides available in the pineapple peels possibly coming from the amylase activity (Evans et al., 2013; Hossain et al., 2015; Sindumathi et al., 2017; Egbune et al., 2021).

Evaluation of the effect of fermentation pH (3 to 9) on the activities of amylase during solid state fermentation by *R. oligosporus* showed a noticeable rise in amylase activity at all the pH values studied when compared with the control (Figure 4). However, marked elevation was noticed at pH 6 (382.2 ± 15.41 µg/g/min). This suggests that weak acidic medium and biomolecules existing in the medium may act as activators which favoured the production of amylase during solid state fermentation of pineapple peels by *R. oligosporus* (Ronda et al., 2015; Casado et al., 2017).

In conclusion, the soluble protein and glucose levels at pH 3 of pineapple peel was improved when subjected to solid state fermentation using the fungus *R. oligosporus.* This work proves that amylase can be produced from solid state fermentation of pineapple peels with *R. oligosporus* particularly at pH 6. Therefore, this agricultural waste could serve as a potential source of raw materials for industrial production of glucose, soluble proteins and amylase.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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