Experimental Study on Citric Acid from Plantain Peel Using Aspergillus Niger

I. INTRODUCTION

Plantains are a staple food in the tropical regions of the world, ranking as the tenth most important staple food in the world and as a staple, plantains are treated in much the same way as potatoes. Since they fruit all year round, plantains are a reliable all-season staple food, particularly in developing countries with inadequate food storage, preservation and transportation technologies. In Africa, plantains and bananas are production are more than twelve million metric tonnes annually in which Nigeria is the lead producer (Ajala et al., 2018). Plantains can be used for cooking at any stage of ripeness, but ripe ones can be eaten raw. As the plantain ripens, it becomes sweeter and its colour changes from green to yellow to black, just like bananas. Green plantains are firm and starchy, and resemble potatoes in flavour. Yellow plantains are softer and starchier yet sweet. Extremely ripe plantains have softer, deep yellow pulp that is much sweeter. Plantains in the yellow to black stages of ripeness can be used in sweet dishes. Steam-cooked plantains are considered a nutritious food for infants and the elderly. A ripe plantain is used as food for infants at weaning, mashed with a pinch of salt.

Processing of plantain into food requires removal of its peels and Nigeria being one of the largest plantain producing countries in Africa (FAO, 2008)[6] which generates a lot of peel waste which are known to constitute a menace to the society thereby adding to the problem of environmental pollution particularly in places where ruminants (sheep and goat) are not allowed to roam about (Idowu et al., 2018).[8] Hence, there is a need to look for alternative way this waste could be eradicated.

Citrnic acid is an organic acid used for acidulants in food industries and Nigeria imports huge amount of it yearly due to national demand. Therefore, this research work will not only provide a solution to the utilization of the waste material but will also increase local production of the citric acid thereby catering for its high demand.

II. MATERIALS AND METHODS

A. Materials

The materials used for the experiments were plantain peels which were collected from the small scale plantain chips manufacturing factory, Kuye Area, Ogbomoso, Oyo state. The peels were dried in tunnel dryer at 60 °C milled and sieved to 10 micro meter particle size. It was packaged in polythene for further analysis for the experiments. Other chemicals used were Sodium Hydroxide, D-glucose, Calcium chloride, distilled water, Benedict reagent, Sodium carbonate and Hydrochloric acid.
B. Determination of Sugar by Acid Hydrolysis of plantain peels

20g of substrate was measured into conical flasks. Conc. HCL and distilled water were added to the substrate to make up 100ml solution. The solution was then placed inside the water bath for heating at varying temperatures. 10g of the solution was weighed and diluted in 100ml of water and filtered. 70ml of distilled water was added to 70ml of the filtrates into a burette. 10ml of Benedict’s reagent and 2g of sodium carbonate was mixed into another conical flask and was titrated while heating on a hot plate magnetic stirrer against the solution in the burette until the mixture changes from light blue to faint green and the titre value was recorded. The sugar content was calculated using the formula

\[
\text{Sugar (g/ml)} = \frac{\text{Titre value} \times 2}{0.32}
\]  

(1)

C. Determination of sugar by Enzyme hydrolysis of plantain peels

About 20 grams of substrate was added to 90 grams of distilled water in a conical flask, and the contents were mixed by vortex. The suspension was autoclave at 121°C and 15 psi for 15 minutes. Then, the suspension was cooled to room temperature, and the pH was adjusted to 6.0 ± 0.05 using calcium hydroxide. Thereafter, 6% (w/w) α-amylase was added, the suspension was heated in a shaking water bath at a pressure of 110 psi at 78°C for 44 minutes, and then the hydrolysate was cooled to room temperature. The pH was adjusted to 5.0 ± 0.05 using calcium hydroxide, 9% (w/w) glucoamylase was added, and the suspension was heated in a shaking water bath at 110 psi at 50°C for 123 minutes. The hydrolysate was then cooled to room temperature. The suspended solids were separated from the liquid by centrifugation. Samples were taken at different time intervals. 70ml of distilled water was added to 70ml of the filtrates into a burette. 10ml of Benedict’s reagent and 2g of sodium carbonate was mixed into another conical flask and was titrated while heating on a hot plate magnetic stirrer against the solution in the burette until the mixture changes from light blue to faint green and the titre value was recorded. The sugar content was calculated using equation.

Sugar content = \frac{\text{Brix value}}{\text{g/l of acid}} \times 10  

(2)

D. Inoculation of Aspergillus niger into the hydrolyzed substrates

The experiments were carried out in batch in conical flask. Standard experiments were conducted in 250ml sterilized flask, each containing 20g of treated cassava peels with 60% moisture content level. Aspergillus niger culture was obtained from Central Research Laboratory of Ladoke Akintola University of Technology, Ogbomosho. This was further sub cultured to generate pure culture of microbes on potato dextrose agar. These cultures were incubated at 32 °C for 72 hours and were then stored on agar slant at 4 °C until needed. It was observed that samples hydrolysed with HCl yielded the highest reducing sugar; therefore they were used in the production of citric acid.

E. Determination of Citric acid of the samples

20g of substrate was measured into 25 conical flask, 0.5ml of conc. HCl acid was added to 99.5ml of distilled water, the mixture was poured into the substrate and mixed thoroughly with spatula, the fungal culture was rinsed with water to become solution, 10ml of the organism concentration was added with 2ml of ethanol to the substrate and placed in the water bath at different temperature and time. 2 drops of phenolphthalein was added into 10ml of the solution in a conical flask and was then titrated against 0.1M NAOH until it turns pink and the titre value was recorded. The percentage yield of citric acid was calculated by

\%

\text{ citric acid} = \frac{\text{Titre Value} \times \text{Acid factor}}{10} \times 100

(3)

Where citric acid factor is 0.0064

F. Determination of pH

The pH was determined with the help of an electronic pH meter. The electrode of the pH meter was dipped into the samples for 1 minute and the pH was recorded. The electrode of the pH meter was rinsed with distilled water after each determination.

G. Statistical Analysis of citric acid yield

The SPSS 20.0 version (2015), a software package was used for statistical analysis. Analysis of variance (ANOVA) was carried out on the data obtained from CA and SAR production earlier described. All the experimental treatments were conducted in three replicates. The experimental data were analysed by using one-way ANOVA and means separation/comparison using Duncan’s multiple range test at 95% confidence level.

III. RESULTS AND DISCUSSION

A. Effect of processing factors on Yield of Reducing Sugars

Effect of temperature on the yield of reducing sugar is as shown in Figure 1. The values of reducing sugar vary from 5.3 to 6.1 g/l as the temperature increased from 50-100 °C. The results show that increase in hydrolysis temperature increased reducing sugar. This is because, at higher temperature, starch in the samples was easily broken down to reducing sugars as earlier asserted by Bekir et al., (2009) [5].
The pattern of sugar yield as affected by sample’s concentration is as shown in Figure 2. The values increased from 5.62 to 7.25 g/l as sample concentration increased from 15-25%. Increase in concentration of the sample directly increased the yield of reducing sugar because more substrates are available for conversion into sugar as the experiment progressed. This observation has earlier reported by Ajala et al., (2019).[2]

Comparison on the potency of acid (HCl) and enzyme (Amylase) on the production of reducing sugar from samples is as displayed in Figure 3. The highest maximum of sugar yielded using acid was 6.3 g/l while maximum amount of sugar yielded using enzyme was 6.98 g/l. In this study, enzyme concentration yielded higher reducing sugar than acid. This observation was earlier reported by Azmi et al., (2017)[4]

Residence time hydrolysis using enzyme and acid is as shown in Figure 4. Residence time using acid used was 20 minutes whereas it took 30 minutes for enzyme hydrolysis; however the yield of reducing sugar using enzyme was 7.73 g/l which was higher than 6.6 g/l in acid. It was observed that when HCl was used to hydrolyse the sample, increase in residence time from 5-15 minutes increased the yield of sugar but reduced afterwards but a reverse case was observed when enzyme was used for the hydrolysis because sugar level decreased from 7.73 to 6.6 g/l from 10 to 30 minutes. That implies that enzyme acted faster on the substrate within the first 10 minutes of the experiment. Hna and Yang (2016)[7] also reported similar observation when Tago starch was hydrolysed with amylase.
B. Effect of processing factors on Citric Acid Production

Inoculum concentration has effect on the yield of citric acid and pH is as shown in Figure 5. As the level of inoculum concentration increased from 7-11%, citric acid increased from 36.5-61.1%. Ajala et al. (2020)[3] reported extensively the influence of inoculum on the yield of citric acid from cassava waste. The pH contrariwise, decreased from 4.4 to 3.8. The decrease in pH implies the substrates yielded more citric acid in the medium.

The pattern of yield of citric acid against fermentation time is sinusoidal in nature. It has two peaks at 24.5 and 96 hours. However, increase in fermentation time increased the yield of citric acid. The pH remained constant through the process.

Ethanol has little significant effect on the production of citric acid as the yield value remained almost constant but the value of pH decreased even with a slight increment in the value of yield citric acid.

IV. CONCLUSION

From the study, it was concluded that increase in the hydrolysis temperature, sample concentration, acid and enzyme concentration all favoured increase in the yield of sugar. Likewise, increase in inoculum concentration and fermentation time increased the yield of citric acid but decreased the pH. However, increase in ethanol concentration had little effect on the yield of citric acid and pH.

REFERENCES


