Production of Citric Acid from Aspergilus Niger

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***Abstract*: - Citric acid is a commercially important product used in several industrial processes. Solid state fermentation was used in this work to produce citric acid by using a locally isolated *Aspergillus niger,* from spoilt solidified pap (eko). About 25g of plantain peel was gelatinized and 100m1 of nutrient solution was added. *Aspergillus niger* spores of about $5x10^8$ was added. The set-up was incubated for 15 days at room temperature. 1ml of the filtrate resulting from the fermentation was taken on 6th day, 8th day and 15th day into a test-tube. Pyridine and acetic anhydride were added and heated in water bath at 32°C for 30 minutes. The absorbance was taken using 420nm as wave length.**

The concentration of the citric acid produced by the locally isolated *Aspergillus niger* on 6th day, 8th day and 15th day of incubation were 4.22g11, 2.29g/1, and 14.77g/1 respectively.

This result, which demonstrate the viability of citric acid production by *Aspergillus niger* isolated from spoilt solidified pap (eko) using plantain peel as a substrate can be of interest to possible future industrial applications.

I. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

1.1.1 The discovery and production of citric acid

Citric acid was first isolated in 1784 by the Swedish chemist, Carl Wilhelm who

crystallized it from lemon juice (Frank et al., 2005). Industrial scale citric acid production began in 1890 based on the Italian citrus fruit industry but in 1893, C.Wehmer discovered that mold could produce citric acid from sugar. However, microbial production of citric acid did not become industrially important until World War I disrupted Italian citrus exports. In 1917, the American food chemist, Jane Currie discovered certain stains of the mold *Aspergillus niger* could be efficient citric acid producers, and the pharmaceutical company PFIZER began industrial-level production using this technique two years later, followed by Citrique Belge in 1929. This production technique is still the major industrial route to citric acid used today. Cultures of *Aspergillus niger* are fed on a sucrose or glucose containing medium to produce citric acid. The source of sugar is corn steep liquor, molasses, hydrolyzed corn starch or other inexpensive sugary solutions (Lotfy et al., 2007). After the mold is filtered out of the resulting solution, citric acid is isolated by precipitating it with lime (calcium hydroxide) to yield calcium citrate salt from which citric acid is regenerated by treatment with sulfuric acid (Lotfy et al ., 2007). Prior to the fermentative process, citric acid was isolated from citrus fruits. The juice was treated with lime (calcium hydroxide) to precipitate calcium citrate which was isolated and converted back to the acid (Frank et al., 2005).

1.1.2 The importance of citric acid

In 2007, worldwide annual production stood at approximately 1,600,000 tonnes. More than 50% of this volume was produced in China. More than 50% was used as acidulent in beverages, some 20% in other food application,20% for detergent applications and 10% for related applications other than food, such as cosmetics, pharmaceuticals and in the chemical industries (Berovic et al .,2007), Some of which has to do with the available data and technology (Ige & Adewale, 2022b) to get valuable insight and also adopting the use of artificial intelligence (Ige & Adewale, 2022a) for future prediction based on changes and predictions of acidic contents in living and non living things.

1.1.3 Citric acid production by fermentation process

Fermentation process is used to produce citric acid which can either be submerged fermentation or solid state fermentation. Submerged fermentation involves liquid substrate while solid state fermentation involves solid substrate. Recently, there has been an increase in number of report on the use of solid state fermentation processes because they exhibit series of advantages over submerged fermentation (Manuel et al., 2010). Solid state fermentation has lower energy requirement, produce less waste water and is environmental-friendly as it resolves the problem of solid waste disposal (Pandey, 2003).

The solid state fermentation and the submerged fermentation differ markedly in terms of the yield of citric acid per gram of substrate. However, while in submerged culture, glucose and fructose were gradually consumed throughout the cultivation, the levels of monosaccharides in solid state fermentation increased slightly during sucrose depletion. (Torrado et al., 2010).

In this research work, solid state fermentation was employed. Plantain peel in powdery ' form was used as a substrate in the fermentation process, nutrient solution was added to it and spore suspension of *Aspergillus niger* was also added.

1.1.4 Plantain peel

This plantain peel consists of carbohydrate, fiber, lignin, galacturonic acid and methoxyl group (Emaga et al., 2010). The powdery form can be gelatinized at 121°C for 20 Minutes and allow to cool. This will aid the *Aspergillus niger* to metabolize the peel as a suitable substrate for citric acid production. The same way the plantain peel is gelatinize before fermentation, so like-wise all other substrates can be gelatinized before fermentation begins.

1.1.5 Aims and Objectives

- To investigate the ability of Aspergillus niger that was isolated from spoilt solidified pap (eko) to produce citric acid.
- To investigate how a local waste product such as plantain peel can serve as a substrate for inoculated Aspergillus niger.

1.2 Literature Review

Vandenberghe et al (2004) produced citric acid by *Aspergillus niger* LPB21 using cassava bagasse as a substrate. They gelatinized the cassava bagasse in order to facilitate starch consumption and the synthesis of citric acid. In their research wok, they used two types of semi-pilot scale bioreactors namely: tray-type and horizontal arum bioreactors. They observed that citric acid production in tray-type bioreactor was 85% while citric acid production in horizontal drum was 69%. They suggested that the citric acid production decreased due to the effect of fermentation temperature which was not controlled in horizontal drum bioreactor. They observed that low respiration rate contributed to the production of high concentration of citric acid.

Qadeer et al (2002) produced citric acid by kinetics of submerged fermentation of *Aspergillus niger* GCBT7 using blackstrap molasses as the fermentation media. They used a laboratory scale stirred fermentor of 15-L capacity for cultivation process and nutritional analysis. Among the 10 stock cultures of *Aspergillus niger* they have, they found that the strain GCBT7 enhanced citric acid production than others. They subjected the strain to parametric studies. They observed that major effects were caused due to oxygen tension (1.0 1/1/min), pH value 6.0 and incubation temperature 30°C. They carried out all fermentation following the growth on 150g/1 raw molasses sugar for 144 hrs. They used ferrocyanide (200ppm) to control the trace metals present in the molasses medium. They added ammonium nitrate to the fermentation media as nitrogen source. They obtained maximum citric acid production of 99.5 ± 3.5g/1 by the *Aspergillus nigger* GCBT7 which was higher than other strains.

1.3 Substrate

Solidified pap (eko) was obtained at Sabo market in Ogbomoso for the: isolation of *Aspergillus niger*. Plantain peel was obtained at starlight area in OgbomOso. It was dried in an oven at 60°C in the laboratory and then grounded to powder at Stadium Road.

II. METHODS

2.2.1 Isolation of Aspergillus niger

The solidified pap was exposed aerobically for minimum of four days. With the aid of a sterile syringe needle, the growth of black spore on the exposed solidified pap (Eko) was picked and inoculated into sterile solidified Potato Dextrose Agar After the inoculation, the plate was incubated in a fungal incubator at room temperature for minimum of three days. After the incubation, black-colored spores and other colored spores were observed.

2.2.2 Sub-culturing

In order to get pure fungal isolates, the black-colored spores known to be fungi were subcultured into another freshly prepared solidified Potato Dextrose Agar, by using a sterile needle.

This plate was incubated at room temperature in a fungal incubator for minimum of three days. After the incubation, a pure black fungal isolates was observed on the sub-cultured plate.

2.2.3 Preparation of slants

680m1 of Potato Dextrose Agar was prepared and poured into MacCartney bottles. The *Aspergillus niger* that produced high yield of suspected citric acid was inoculated into each slant in the McCartney bottles.

2.2.4 Screening for citric acid producing strain of A. niger

The *Aspergillus niger* strains were screened quantitatively in petii-plate for the production of citric acid. The selected media for screening of citric acid producer was Czapek-Dox Agar with Bromocresol blue as the indicator. Czapek-Dox Agar with Bromocresol blue medium 15m1 was poured into individual sterile petri-plate and allowed to cool at room temperature. Each *Aspergillus niger* culture was transferred with sterile needle to each of the petri-plate and labeled. The plates were incubated at 25°C for three to five days. The plates were observed after incubation for yellow zone due to citric acid formation. The strain of Aspergillus niger with the widest yellow zone was used for further studies. The plate is shown in figure 1.

2.2.5 Substrate treatment

The substrate used was grinded plantain peel. About 25g of this substrate was put in a Bama-bottle and was thermally treated to gelatinize the starch by adding 1, 100m1 of distilled water into it. The substrate was heated for 20 minutes at 121°C in an autoclave. This heated substrate is termed gelatinized substrate.

2.2.6 Preparation of nutrient solution

About 1000m1 of nutrient solution was prepared. The nutrients involved are : Urea(2.93g), Potassium Phosphate (1.86g) and Iron Sulphate Pentahydrate (0.0105g). The nutrient solution was sterilized in an autoclave and when it cooled, 40m1 was deducted from it and was replaced with 40m1 of sterilized 4% methanol. 60m1 from the resulting solution was inoculated into the plantain peel in the bama-bottle explained in section 2.2.5.

2.2.7 Preparation of saline solution (0.9% sodium chloride)

About 1000m1 of saline solution was prepared by adding l0g of sodium chloride salt into 990m1 of water. The solution was sterilized for 20 minutes at 121°C in an autoclave.

2.2.8 Preparation of spore suspension

The spore suspension of *Aspergillus niger* was prepared by washing the five-day old culture slant with 20m1 of sterilized saline solution of 0.9% sodium chloride. 2m1 of the spore suspension was incubated into the gelatinized plantain peel in the bama-bottle and was incubated at room temperature for 15days.

2.2.9 Commercial citric acid estimation

1 molar citric acid solution was prepared according to manufacturer's instruction; this was noted as stock solution. From this, 7 standard concentrations were prepared as indicated in the table below;

Table 1: Showing commercial citric acid estimation.

Standard concentrations	Molar concentration	Absorbence
First standard concentration	0.01	0.011
Second standard concentration	0.02	0.014
Third standard concentration	0.04	0.018
Fourth standard concentration	0.05	0.017
Fifth standard concentration	0.07	0.021
Sixth standard concentration	0.08	0.02
Seventh concentration	0.1	0.014

1 ml from each of the seven standard concentrations was extracted into different seven test-tubes. To 1 ml of the standard solutions, 1.3m1 of pyridine and 5.7 ml of acetic anhydride were dropped into them each. The test-tubes were placed in a water-bath at 32°C for 30 minutes. The absorbance of each treated standard solution was taken by using a UV-Spectrophotometer at a wavelength of 420nm. The result obtained was used): to draw a straight line graph of absorbance against concentration (Figure 2). With this curve, the straight line equation below was generated. This straight line equation was used to determine the concentration of fermented citric acid.

X = Y — 0.013/0.055…….Equation 1

Where X = concentration of fermented citric acid (g/1)

Where Y = absorbance of fermented citric acid.

2.3.0 Extraction of fermented citric acid

After the completion of fermentation process, the incubated culture was filtered for the separation of pellet form of fungal culture and the fermentation broth, which acts as the source of citric acid. Calcium hydroxide was added to the fermentation broth to allow precipitation of citric acid in the form of calcium citrate. Again, the precipitate was treated with dilute tetraoxosulphate (vi) acid to precipitate insoluble calcium sulphate, and then filtered. The resulting fermented citric acid was then collected into a bama-bottle and kept in a refrigeration.

III. RESULT

Fermentation process was carried out for citric acid production. During the fermentation process, the room temperature was maintained up to the end of the process. The concentration of the raw fermented citric acid produced by the indigenous *Aspergillus niger* on 6th day, 8th day a and 15th day of incubation was 4.22g/l. 2.29g/1, and 14.77g/1 respectively.

3.1 Fermented citric acid estimation

The fermented broth was filtered by using filtered paper in order to remove the mycelia of the *Aspergillus niger.* 1 ml of the filtered culture was put in a test-tube with the addition of 1.3m1 of pyridine and 5.7m1 of acetic anhydride. The test-tube was placed in water-bath for 30 minutes at 32°C and the absorbance was taken at 420nm as wavelength. This was done on the 6th day, 8thday, and 15th day of fermentation.

Fermented citric acid concentration using equation 1:

X = Y — 0.013/0.055……Equation 1

Where X = concentration of fermented citric acid (g/1)

Where Y = absorbance of fermented citric acid.

The results obtained are shown in the table below;

Table 2: Yields of Citric acid at different Sampling days of fermentation.

Fermentation days	Yields of Citric acid (g/L)
Day 6	4.22
Day 8	2.29
Day 15	14.77

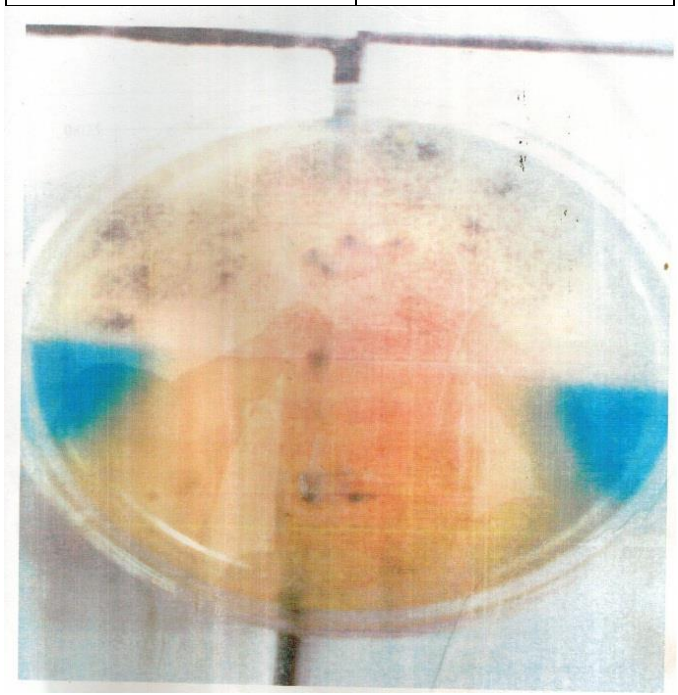

Figure 1: A petri-plate showing citric acid producing strains of *Aspergillus niger*.

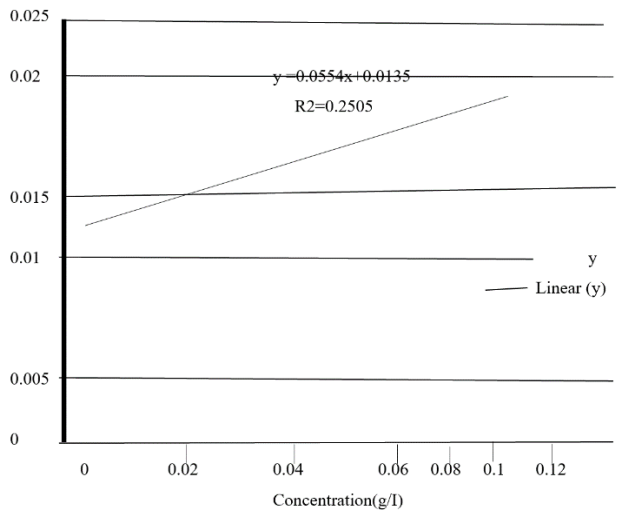

Figure 2: A graph of absorbance against concentration of commercial citric acid

IV. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

This research work has demonstrated the ability of a locally isolated *Apergillus niger* that was isolated from spoilt solidified pap (eko) to produce citric acid by using plantain peel as a substrate. From this research, it is evident that plantain peel can be used as a substrate for *Aspergillus niger* to produce considerable yield of citric acid.

4.2 Recommendation

From the result obtained in this research work, I thereby recommend the following:

- I recommend that agro-wastes such as plantain peel should be used as a substrate for citric acid production instead of using chemically synthesize citric acid. The use of this waste prevents environmental pollution.
- I recommend that in subsequent analyses, detail characterization of the *Aspergillus niger* should be done.
- Also, a pH meter should be used to determine the pH of the citric acid that is produced daily by the *Aspergillus niger* during the fermentation period

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