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OPTIMIZATION PRODUCTION OF CITRIC ACID BY *Aspergillus niger* THROUGH SUBMERGED FERMENTATION OF DRIED ORANGE PEELS FROM GONIN-GORA, CHIKUN L.G.A., KADUNA STATE, NIGERIA

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ABSTRACT

Citric acid is commercially produced through submerged fermentation using microorganisms like *Aspergillus niger* on various substrates. This study produced citric acid from orange peels through submerged fermentation using *Aspergillus niger* at varying incubation times with the addition of a nitrogen supplement and the percentage yields for the samples used were compared. The results showed that incubation time and nitrogen supplements (ammonium nitrate) can increase the citric acid production with *Aspergillus niger*. The maximum citric acid concentration obtained for the sample with a nitrogen supplement (Sample B) was 6.14816 gL^{-1} (1g ammonium nitrate) for an incubation time of 168 hours while the maximum citric acid concentration obtained for the sample without a nitrogen supplement (Sample A) was 4.99538 gL^{-1} for an incubation time of 96 hours.

Keywords; *Aspergillus niger*, Citric acid, Submerged fermentation

INTRODUCTION

Citric acid is a weak organic acid that has the chemical formula $C_6H_8O_7$ and IUPAC name 2-Hydroxypropane-1,2,3-tricarboxylic acid. It is found naturally in citrus fruits, especially lemons and lime; it is what gives them their sour taste. It is an odorless crystalline white solid. It is considered among valuable commercial organic acids which are used in food, beverage, and pharmaceutical industries [1]. It has many applications in food, pharmaceutical and cosmetic industries as an acidulant, flavor enhancer, colorant, preservative, buffer, antioxidant, emulsifier, and chelating agent [2]. More than two million metric tons of citric acid is manufactured every year.

Citric acid exists in greater than trace amounts in a variety of fruits and vegetables, most notably citrus fruits. Lemons and limes have particularly high concentrations of the acid; it can constitute as much as 8% of the dry weight of these fruits (about 47 g/l in the juices) [3]. The concentrations of citric acid in citrus fruits range from 0.005 mol/L for oranges and grape fruits to 0.30 mol/L in lemons and limes; these values vary within species depending upon the cultivar and the circumstances in which the fruit was grown.

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Orange peel contains soluble sugars and pectin as the main components. The orange peel is in fact constituted by soluble sugars, 16.9 % wt; starch, 3.75 % wt; fiber (cellulose, 9.21 % wt; hemicelluloses, 10.5 % wt; lignin 0.84 % wt; and pectins, 42.5 % wt), ashes, 3.50 % wt; fats, 1.95 % wt; and proteins, 6.50 % wt [4].

Agricultural waste is an abundant, inexpensive and renewable material for the production and sustainability of valuable organic acids such as citric acid. The increased demand for citric acid by industries coupled with the search for an efficient raw material from agricultural waste residue for its production is of great concern [5].

Citric acid can be produced through chemical processes, but fermentation processes using the fungi *Aspergillus niger* are still preferred to commercially produce citric acid because of its ease in handling, ability to utilize a variety of substrates and produce large amount of citric acid. More than 90 % of the citric acid produced in the world is obtained by fermentation because the operations are simple and stable. Citric acid production by fermentation can be divided in three phases, preparation and inoculation of the raw material, fermentation, and recovery of the product. The production of useful products like citric acid from biomass is influenced by fermentation parameters like incubation period, substrate, substrate concentration, inoculum size, moisture content, pH, and temperature [6]. The submerged technique is widely used for citric acid production. It is estimated that about 80 % of world production is obtained by submerged fermentation [7]. Submerged fermentation can be carried out in batch, fed batch or continuous systems, although the batch mode is more frequently used. Normally, citric fermentation is concluded in 5 to 12 days, depending on the process conditions [8].

Many agricultural wastes have been explored for the production of citric acid. Orange peels are waste generated mainly by the consumption of orange fruits and after consumption these fruit peels can lead to environmental pollution as solid waste due to its large volume of generation. The high consumption of oranges and physico-chemical characteristics of waste peels can cause soil and water pollution if not properly managed. For this reason, the recovery and transformation of orange peels has become a topic of interest for recent investigations. The waste materials of the orange fruit is made up of mainly of peels, shells, seeds. Orange peels have a high level of organic matter and low pH; such indicators demonstrate the potential polluting agent.

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Maps of the sampling area

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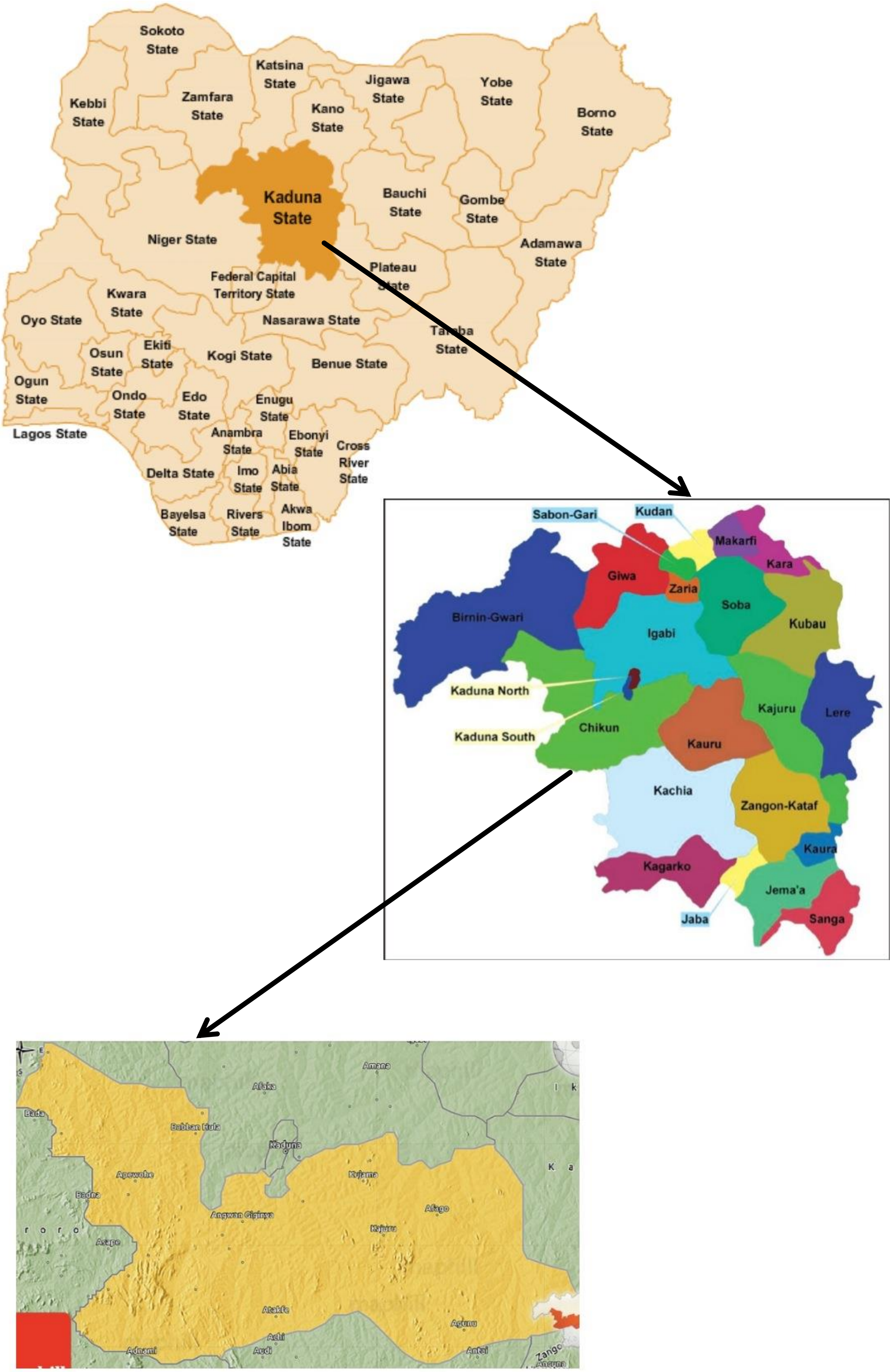

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MATERIALS AND METHODS

Sampling

The raw material (orange fruits) was obtained federal housing estate Gonin-gora, Kaduna State. The fruits were washed with clean running water to remove dirt and debris and the peels separated from the fruits, cut into small pieces and dried under direct sunlight for two weeks to reduce the moisture content and ground into powder using a mortar and a pestle and homogenized in a single lot to avoid any variation in composition, and stored in an airtight container until ready for use.

Citric acid production by submerged fermentation

Two samples of orange peel powder (Sample A and Sample B) were subjected to submerged fermentation, one supplemented with ammonium nitrate and the other without and the results compared

Preparation of spore suspension

The fungi spores of *Aspergillus niger* was gotten from the department of microbiology, Kaduna State University, the spores were put in a petri dish and wrapped with foil paper to avoid cross contamination. The fungal spore suspension was prepared by dispersing spores in peptone water and the peptone water was prepared by suspending 25 grams of peptone salt in 1000ml of purified or distilled water and was heated to dissolve the medium completely. It was dispensed in tubes and sterilized by autoclaving at 15lbs pressure (121^{0}C) for 15 minutes.

Preparation of fermentation medium

65grams of sabouraud dextrose agar was dispersed in 1000ml of distilled water and stirred until the solid particles dissolved.

For sample A

30g of powdered orange peel was dispensed into an Erlenmeyer flask (500 mL) and 300 mL of sabouraud dextrose medium added into the flask. The mixture was autoclaved at 121^{0}C for 15 min and allowed to cool to room temperature and the P^H of the medium adjusted to 6.5 using hydrochloric acid solution, the flasks were then inoculated with 1 mL of fungal spore suspension and incubated for 7 days at a temperature of 28±2^{0}c

For sample B

30g of powdered orange peels was dispensed into an Erlenmeyer flask (500 mL) and add 300 mL of sabouraud dextrose medium into the flask. The mixture was autoclaved at 121^{0}C for 15 min to sterilize and allowed to cool to room temperature, the P^H of the medium was adjusted to 6.5 using hydrochloric acid solution and supplemented with 1g of ammonium nitrate and then inoculated with 1 mL of fungal spore suspension and incubated for 7 days at a temperature of 28±2^{0}c.

Determination of citric acid

Citric acid of both sample A and B was determined starting from day 4 of the incubation

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all through to day 7 of incubation and the acidity and percentage yield calculated for each day.

Citric acid was determined titrimetrically, in this procedure, 10mL of the culture filtrate was taken into a flask and then 2 drops of phenolphthalein indicator will be added and titrated with 0.1M NaOH. The end point will be noted when the filtrate changes from colorless to pink color.

The acidity was calculated as:

$$\text{Acidity} = \frac{\text{TV x } 0.007005 \text{ x } 100}{\text{Volume of sample}}$$

Where; TV= titre value

Standard acidity factor for citric acid = 0.007005

Yield calculation

The Citric Acid yield was calculated using the formula:

$$\text{Citric acid } (gL^{-1}) = \frac{192.13 \times M \times V}{\text{Weight of sample}}$$

Where;

192.13 $gmol^{-1}$ = Molar mass of citric acid

M = Molarity of NaOH

V = Volume of NaOH used during titration

RESULTS AND DISCUSSION

Citric acid of both sample A and B was determined starting from 96 hours of the incubation all through to 168 hours of incubation and the acidity, concentration and percentage yield calculated for each day and the results shown in the following tables.

Citric acid production has been shown to be viable with many cheap agricultural raw materials (Pawar and Pawar, 2014) and this was also obtained in this experiment as both samples managed to produce citric acid. With reference to the table above, the highest maximum citric acid yield was 4.99538 gL^{-1} (96 h) and 6.14816 gL^{-1} (168 h) for sample A and B respectively, with sample B being the better of the two.

Table 1: Acidity of Citric Acid produced from submerged fermentation of dried orange peels from Gonin-gora from 96-168 hours of incubation

SAMPLE	96 HOURS	120 HOURS	144 HOURS	168 HOURS
A	0.18213%	0.175125%	0.16812%	0.147105%
B	0.07005%	0.133095%	0.18213%	0.22416%

Table 2: Yield of Citric Acid produced from submerged fermentation of dried orange peels from Gonin-gora from 96-168 hours of incubation

SAMPLE	96 HOURS	120 HOURS	144 HOURS	168 HOURS
A	4.99538 gL^{-1}	4.80325gL^{-1}	4.61112 gL^{-1}	4.03473gL^{-1}

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B	1.9213gL^{-1}	3.65047 gL^{-1}	4.99538 gL^{-1}	6.14816 gL^{-1}

Effect of nitrogen

Nitrogen has a profound effect on citric acid production as it is not only important for metabolic rates in the cells but is also a basic part of cell proteins and was shown to induce pellet formation in filamentous fungi [9]. Nitrogen has been reported to be an important factor in fermentation processes due to an increase in C/N ratio [10],[11]. This experiment showed that nitrogen influences citric acid production using *A.niger* as shown by the table 2 above where the maximum citric acid production of sample B supplemented with 1g ammonium nitrate was 6.14816 gL^{-1} after 168 hours which is in agreement with [12] who recorded maximum citric acid production of 5.22 gL^{-1} after 168 hours. [13] showed that the effect of the nitrogen on citric acid production varied with the specific type of nitrogen source used. In this study it was seen that sample B which was supplemented with ammonium nitrate (6.14816 gL^{-1} at 168 h) had the highest citric acid production as compared to sample A (4.99538 gL^{-1} at 96 h) which agreed with [9] that nitrogen has a profound impact on citric acid production.

Effect of incubation time

The table above illustrates the impact of fermentation period on citric acid yield after 168 hours of incubation at 28±2^{0}C. At 0 hours, citric acid was not produced, but as fermentation period increased to 96 hours, citric acid production increased significantly to for sample A and 1.9213gL^{-1} for sample B. sample A gave the highest yield of citric acid after 96 hours (4.99538 gL^{-1}) and after that decrease in citric acid production as shown in table 2 while sample B had maximum citric acid production at 168 hours with a significantly high yield of 6.14816 gL^{-1}. At 96h and 144h for sample A and B respectively equal citric acid yield was recorded (4.99538 gL^{-1})

The optimum incubation period for maximum citric acid production for sample A was 96hours which differs from the findings of [14] in which maximum citric acid production occurred at 168 hours of incubation and for sample B was 168 hours which is in agreement with [12] who reported maximum yield after 168 hours. Sample B showed a constant increase in citric acid production as the incubation time increased, the production begins to increase after a lag phase of approximately 2-3 days and reaches maximum at the stationery phase [15] and this observation corroborated with [16] in which high citric acid yield occurred at 168 hours of fermentation but differs with the findings of [17] and [18] in which high citric acid yield occurred at 192 hours of fermentation.

CONCLUSION

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In conclusion, citric acid was produced from orange peel waste successfully using *A.niger*. This study also showed that incubation time and nitrogen supplements (ammonium nitrate) can increase the citric acid production with *A.niger*. The maximum citric acid concentration obtained for the nitrogen supplements was 6.14816 gL^{-1} (1g ammonium nitrate) and for incubation time was 4.99538 gL^{-1} (96 hours) for sample A and 6.14816 gL^{-1} (168 hours) for sample B. The success of orange peel a as substrate for citric acid production using *A.niger* in this study under submerged fermentation has shown that agro-waste can be an inexpensive substrate for the production of citric acid and further studies should be carried out on various agro-wastes and their capabilities of producing this valuable acid. At the moment in Nigeria, little effort is being made to promote industrialization and the use of cheap substrates for the production of various consumables and the most promising solution to reduce cost and increase profit is the utilization of the indigenous micro-organisms and cheaper agricultural wastes (such as waste orange peels) as substrate for the production of this valuable organic acid and other products as well.

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