

## EFFECT OF SUGAR SUPPLEMENT ON CITRIC ACID YIELD ABILITY OF AN INDIGENOUS *ASPERGILLUS NIGER* USING KOLANUT POD AS FEEDSTOCK

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### ABSTRACT

The effect of different concentrations of sugar (sucrose, glucose, fructose and galactose) as supplement on kolanut pod used as substrate for citric acid production was investigated. A locally isolated *Aspergillus niger* from spoiled orange fruit was used for the production of citric acid (CA) using kolanut pod wastes, as substrate. The dried pulverized substrates of kolanut pod were supplemented with different concentrations 5, 10, 15% (w/v) of sucrose, glucose, fructose and galactose. Samples from the fermenting substrate were taken at every 48 hours interval while the citric acid yield was estimated spectrophotometrically using pyridine-acetic anhydride method. It was observed that the substrate fortified with 15% (w/v) sucrose gave the highest amount of concentration (31.67 g/l) of CA. Both 5% and 10% (w/v) sucrose concentration has no level of significant difference ( $P < 0.05$ ). A low yield of CA (2.43 g/l) was recorded from Kolanut Pod alone. Significantly lower values were obtained using the same concentration of other sugars as compared to sucrose. Multiple comparison Test was used to analyse the variance of the entire sugar mean. Similarly, there was a significant difference in the 15% (w/v) concentration of sucrose with the higher mean of 15.21 g/l produced after day 20. Therefore, the effects of various sugar concentrations as a supplement to boost the rate at which indigenous *Aspergillus niger* produces citric acid could serve as an approach to citric acid optimization in future biotechnological process.

**Key words:** Citric acid, *Aspergillus niger*, Kolanut pod, surface culture fermentation, Optimization

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### INTRODUCTION

Citric acid is a commercially valuable chemical commodity. It is commercially produced by submerged fermentation (SmF) of starch or sucrose based media (sucrose or glucose syrups) using *Aspergillus niger* (Lofty *et al.*, 2007; Barrington and Kim, 2008). Industrially, citric acid finds applications in the manufacture of food and beverages, pharmaceuticals, chemical and cosmetics. Far above 70% of total citric acid produced globally is consumed by food industry while 12% is used by pharmaceutical industries and the rest 18% by other industries (Nadeem *et al.*, 2010; Gueguin-Kana *et al.*, 2012). It is generally used in industries for acidulation, anti-oxidation, emulsification, preservation, flavour enhancement and as plasticizer and synergistic

agent (Soccol *et al.*, 2006; Bhattacharjee and Baruah, 2015).

There is a high global demand for the consumption of this organic acid due to its low toxicity when compared with other acidulants (Vasanthabharathi *et al.*, 2013). At the moment, the global demand for citric acid is growing faster than its production as estimated by Business Communications Co. (2011) (BCC, [www.bccresearch.com](http://www.bccresearch.com)) while the cost of existing raw materials is increasing thus requiring an alternative economical process with new substrates as attractive over the SmF process (Anastassiadis *et al.*, 2008; Gueguin-Kana *et al.*, 2012). In Nigeria, the entire demand for citric acid is met by import (Betiku *et al.*, 2010). Therefore, to meet up with the growing demand of this metabolite (citric acid)

an economical process of its production is needed which is fermentation process.

Microbial fermentation have been reported to be the oldest process known to man for making variety of value added products such as citric acid (Akpan *et al.*, 2005; Angumennal and Venkappayya, 2013). Moreover, fermentation process has numerous advantages as it is very mild and ecofriendly which consumes less energy (Angumeenal and Venkappayya, 2013). Among large array of microorganisms that have have been reported in the production of citric acid, *A. niger*, a common black mold remains the preferred choice for citric production due to its ease of handling (Nadeem *et al.*, 2010). More so, it has ability to ferment a variety of raw materials (Kareem *et al.*, 2010; Sukesh *et al.*, 2013) and more importantly owing to its well-developed enzymatic system which enhances higher yields as compared to other micro-organisms (Vandenbergh *et al.*, 1999; Bhattacharjee and Baruah, 2015).

Several by-products and residues of agriculture and agro-industries have been employed as raw materials in the production of citric acid (Pandey *et al.*, 2000; Mussatto *et al.*, 2012). An attempt is made in this work to utilize the kolanut pod waste fortified with differnt concentrations of sugars (sucrose, glucose, fructose and galactose) as substrate using an *A. niger* for citric acid production. Kolanut (a member of the family Sterculiaceae) is mostly produced in Africa and is cultivated to a large extent in Nigeria which accounts for about 70% of the total world production (Azogwa *et al.*, 2011). Kolanut pod is a by-product generated during extraction of the nut from the pod. It is renewable and abundant locally. Apart from its being used for animal feeds, it is generally treated as a waste and disposed in the sewage system. Therefore, considering its low cost, high carbohydrate content and susceptibility to fermentation have made kolanut pod, an attractive raw material for CA biotechnological production.

## MATERIALS AND METHODS

### 1. Isolation and identification of *Aspergillus niger* from spoilt orange

Black mycelium of *Aspergillus niger* used in this work was isolated from naturally decomposed orange fruit pulp using a sterile needle and prepared according to the method described by Sani *et al.* (1992). The fungal cultured was grown on a Potato Dextrose Agar (PDA) and incubated at  $28 \pm 2$  °C for 120 hrs.

### 2. Screening of the isolate used for citric acid production

The strains of *A. niger* were screened qualitatively in Petri dish for the production of citric acid using Czapek–Dox agar, supplemented with 5% bromocresol-green as indicator. The medium was poured into individual sterile Petri plates and maintained at  $28 \pm 2$  °C. Each plate was inoculated with *A. niger* with sterile inoculating needle and labelled. The plates were incubated for 5 days at room temperature, and observed for the development of yellow zone which indicates citric acid production (Rao and Reddy, 2013). Spores that produced the largest yellow was picked for further studies. Spores which formed yellow zone were washed out twice with 10 ml sterile distilled water each time (Plate 1). Spore suspensions containing about log 8/ml were prepared and used as inoculums for the fermentation process (Ali *et al.*, 2004).

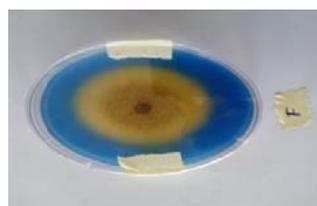


Plate 1: *A. niger* exuding yellow zone during screening for citric acid production

### 3. Substrate Gelatinization and Thermal Treatment

Kolanut pod waste used as substrate were procured from Ifon town (Latitude: 7.86667 and Longitude 4.48333) in Osun state, Nigeria and transported to the laboratory in an aseptic polythene bag. The substrates were sundried to reduce the moisture content and pulverized to a

very small size (2mm). The substrate was thermally treated according to the method described by Vandenberghe, (2006).

#### 4. Basal Fermentation Media

Fermentation process was carried out in a 250 ml Erlenmeyer flask containing Kolanut pod substrate fortified with different concentrations 5, 10, 15% (w/v) of sucrose, glucose, fructose and galactose and supplemented with nutritive solution containing (g/L) 2.93 urea, 1.86  $\text{KH}_2\text{PO}_4$ , 0.0105  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and 4% methanol which was then sterilized at  $121^\circ\text{C}$  for 15 minutes. This fermentation medium was then inoculated with spore suspension and incubated at  $30^\circ\text{C}$  for 20 days.

#### 5. Experimental set up

Twenty five grams (25g) of gelatinized substrate were dispensed into 250 ml of Erlenmeyer flask provided with rubber caps, 60 ml of nutrient was then added and fortified with 100 ml of a known sugar concentration and then sterilized together. After cooling, the media was inoculated with 2 ml spore suspension and was incubated at room temperature for 20 days.

#### 6. Assay of citric acid

The concentration of citric acid in the fermentation medium was determined by using pyridine-acetic anhydride method according to Marrier and Boulet (1958). The absorbance of the reaction mixture at 420 nm was determined using a UV-Visible spectrophotometer (Spectrumlab A 23). The citric acid content of the samples were extrapolated with reference to the standard equation  $Y=0.0554 x + 0.0135$ , where Y is the absorbance; x is the concentration of citric acid yield [g/l]; gradient (M) = 0.0554 and the intercept (C) = 0.0135

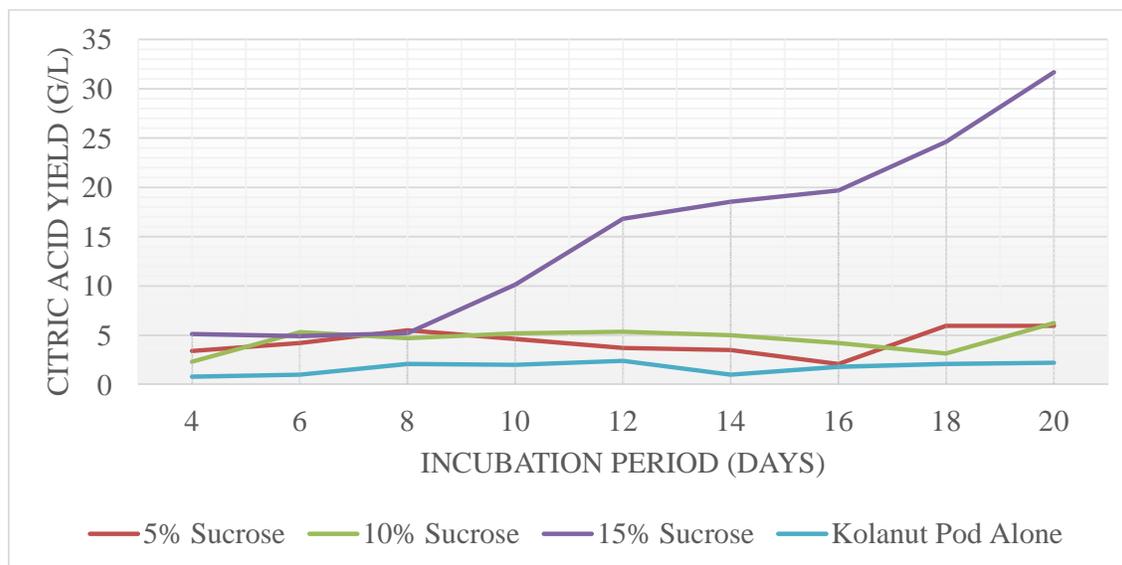
### RESULTS AND DISCUSSION

Presented in Fig. 1, 2, 3 and 4 are CA yield by indigenous *A. niger* inoculated in medium of Kolanut pod as a basic fermentation media fortified with different concentrations of sucrose, glucose, fructose and galactose. Significantly lower concentration of CA (2.41g/l) was produced from Kolanut pod alone

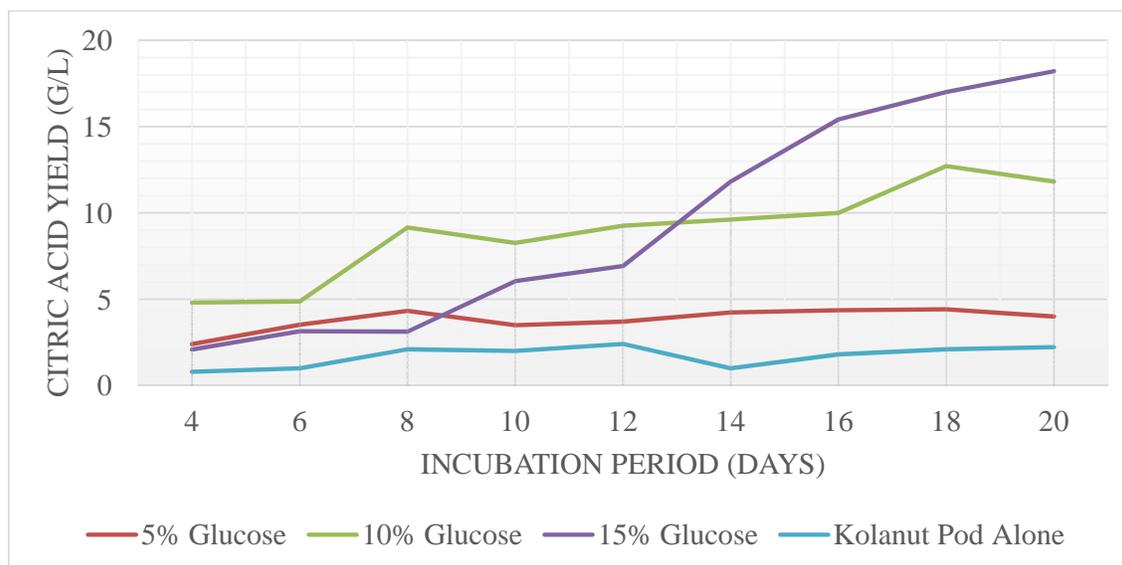
after 20<sup>th</sup> day of incubation. Moreover, with different concentrations of sugars added to the Kolanut pod, CA production was enhanced. It was observed as shown in Fig. 1 that the 15% sucrose rich substrate gave the maximum production of CA (31.67g/l) realised on the 20<sup>th</sup> day of fermentation. The indigenous *A. niger* was able to ferment the disaccharide sugar into citric acid. The yield obtained from the kolanut pod supplemented with 15% sucrose concentration appeared higher than any of the result obtained from the different concentrations of other sugars investigated in this work. This result shows similarity with the report of El-Holi and Al-Delaimy (2003) that reported maximum value of 106.5 g/L of CA from whey supplemented with 15% sucrose. Yakoya, (1992) opined that citric acid accumulation is strongly influenced by the type and concentration of carbon source. Therefore carbon source for citric acid production is of utmost importance. Hossain *et al.* (1984) opined that sucrose is preferable to glucose, fructose and galactose. Moreover, sucrose is of relatively low molecular weight and is readily transported into microbial cells for hydrolysis by intracellular enzymes (Drysdale and McKay, 1995). Max *et al.* (2010); Nadeem *et al.* (2010) reported that *A. Niger* possesses an extracellular mycelium-bound invertase that is active at low pH. More so, sucrose has been described as the traditional commercial substrates for CA production.

Moreover, lower values of CA were obtained when this same concentration of other sugars (glucose, fructose and galactose) were added given 18.21, 10.0 and 15.11 g/l respectively as shown in Figs. 2, 3 and 4. Moreso, with 5% fortification of the different sugar supplement, low yield of CA were recorded all through.

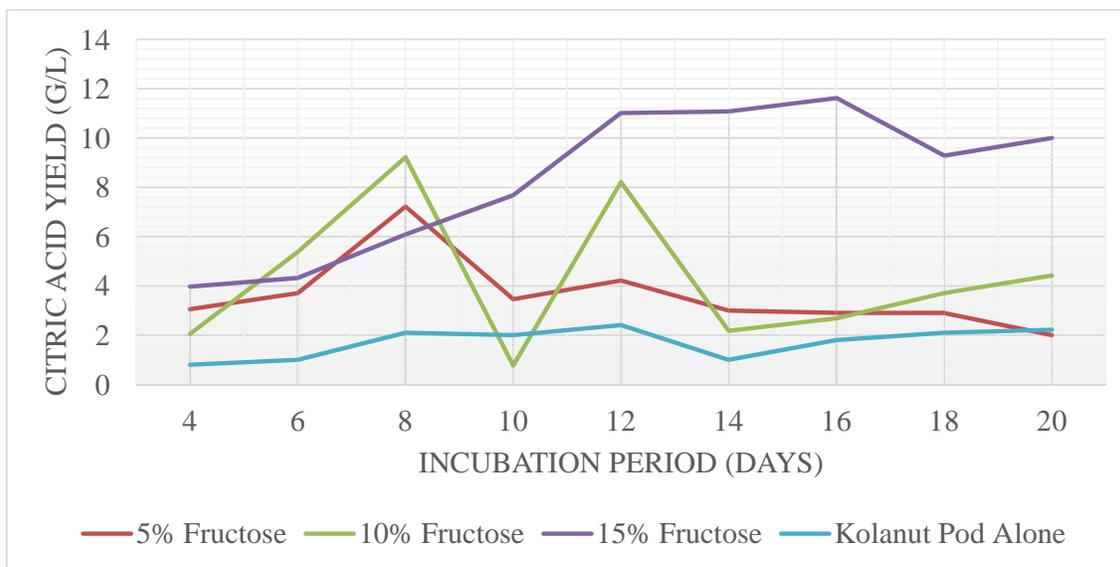
With low yield of citric acid accounted for when kolanut pod alone was fermented this could be attributed to the carbohydrate content of the pods. Dolan and Mankey (2005) equally reported that the concentrations and type of raw materials are also important parameter for citric acid production.



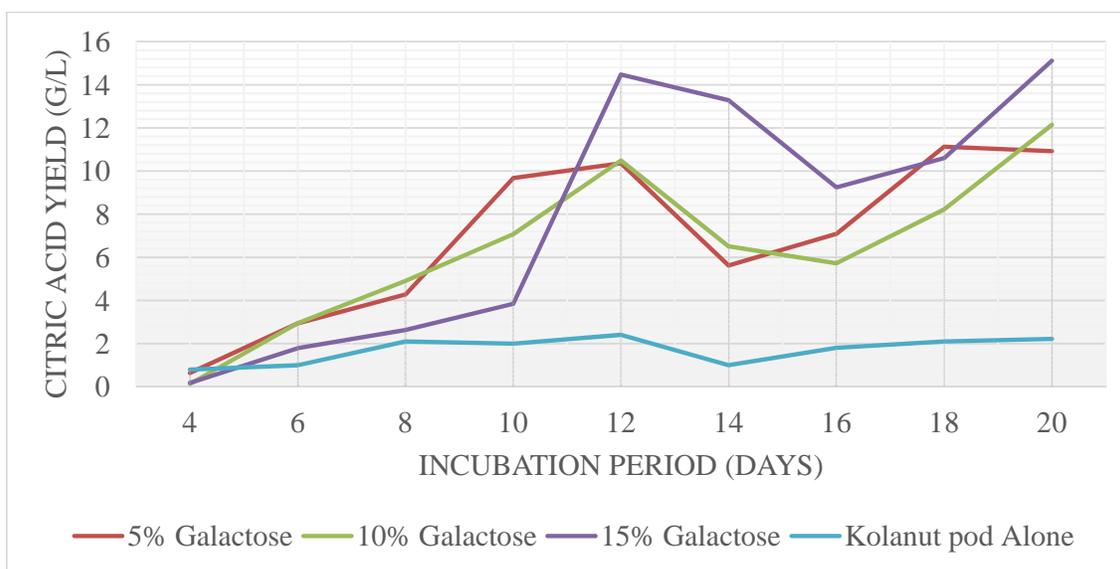
**Figure 1:** CA yield by *A. niger* inoculated in medium of Kolanut pod medium fortified with different concentrations of sucrose



**Figure 2:** CA yield by *A. niger* inoculated in the medium of Kolanut pod medium fortified with different concentrations of glucose



**Figure 3:** CA yield by *A. niger* inoculated in medium of Kolanut pod medium fortified with different concentrations of fructose



**Figure 4:** CA yield by *A. niger* inoculated in medium of Kolanut pod fortified with different concentrations of galactose

## CONCLUSION

In conclusion, this study has been able to show the prospect of kolanut pod starch as an alternative carbon source when fortified with a nutritive solution of different concentrations of various sugars for the production of CA by indigenous *A. niger*. Therefore using kolanut pod with nutritive solution alone as a fermentation medium for CA production cannot be compared to the fortified kolanut pod with sugars. However, more work still need to be carried out on the optimization of the process via fermentation conditions and other fermentation types.

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