

Available online on 30.08.2019 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Utilization of Fruit Waste for the Production of Citric Acid by using *Aspergillus Niger*

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ABSTRACT

The main organic acids in industrial used were citric, acetic, tartaric, malic, lactic and gluconic acid. The most utilized organic acid is citric acid or tricarboxylic acid. Chemical synthesis of citric acid is more costly than fermentation. The citrus processing industry generates tons of waste such as peel and segment membranes resulting from the extraction of citrus juice in industrial plants. About 6 isolates were obtained from the fruit waste dumped soil and they were subjected to screening for citric acid production. *A. niger* over other potential citric acid-producing organism towards efficient utilization of agroindustry residues and by-products for citric acid production. It was grown on PDA plates at a temperature of 28°C for 5 to 7 days. Fruit wastes like orange peel, sweet lime peel, banana peel and pineapple peel as a potential substrate for the production of citric acid using *A. niger* isolates. The nitrogen source of the fermentation medium will have a direct effect on the yield of citric acid. Among the four fruit wastes, citric acid production was maximum from orange peel followed by sweet lime peel, pineapple peel and banana peel respectively. Orange peel contains soluble sugars and pectin as the main components. Study the impact of different carbon sources on citric acid, the basal media were supplemented with glucose, sucrose, fructose, maltose and the fermentation were carried up to 5 days at 30°C. Nitrogen sources on the fermentation were studied by incorporating ammonium phosphate, potassium hydrogen phosphate and peptone. The effect of lower alcohol incorporation on the fermentation of citric acid was studied.

Article Info: Received 09 July 2019; Review Completed 21 Aug 2019; Accepted 25 Aug 2019; Available online 30 Aug 2019**Cite this article as:**

S. Satheeshkumar, P. Sivagurunathan, K. Muthulakshmi, C. Uma, Utilization of Fruit Waste for the Production of Citric Acid by using *Aspergillus Niger*, Journal of Drug Delivery and Therapeutics. 2019; 9(4-A):9-14
<http://dx.doi.org/10.22270/jddt.v9i4-A.3487>

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INTRODUCTION

The citric acid (2-hydroxy-propane 1, 2, 3-tricarboxylic acid) derives its name from the Latin word citrus, the fruit of the citrus tree which resembles a lemon. Citric acid is a tricarboxylic acid with a molecular weight of 210.14 Da¹. With an estimated annual production of about 10,00,000 tons, citric acid is one of the fermentation products with the most elevated level of production world wide². A considerable amount of citric acid is required in several industrial processes³. The food industries consume about 70% of the total citric acid production, while other industries (Pharmaceuticals, Beverages, etc) consume the remaining 30%⁴. Chemical synthesis of citric acid is more costly than fermentation. Various kinds of yeast, fungi and some bacteria are known to produce citric acid⁵. The reason for choosing *A. niger* over other potential citric acid-producing organism are cheap raw materials used as substrate; high consistent yields. There has been an increasing trend towards efficient utilization of agro-industrial residues and by-products for citric acid

production⁶. India positions sixth place in the production of citrus fruits in the world and the citrus fruit wastes are also generated in million tons for every year⁷. The citrus processing industry generates tons of waste such as peel and segment membranes resulting from the extraction of citrus juice in industrial plants. The management of these wastes, which produce odor and soil pollution, represents a noteworthy issue for the food industry⁸.

Orange peel contains soluble sugars and pectin as the main components. According to⁹, the orange peel is constituted by soluble sugars, starch, 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% wt¹⁰. Peels of sweet lime contain a higher concentration of phenolics, flavonoids, ascorbic acids, carotenoids and reducing sugars¹¹. Pineapple peel is a by-product resulting from the processing of pineapple into slices and represents about 10% (w / w) of the weight of the original fruit. Pineapple peel is rich in cellulose, hemicellulose and other carbohydrates¹².

Banana is one of the most important major fruit crops grown in India. In respect of area and production, it ranks second only to mango. The banana culture in India is as old as Indian civilization. The peel of banana was a rich source of water, sugar, protein, fat, fiber, tannin, carotene, pigments¹³. Due to the impact of dumping fruit wastes into the environment and about the wide applications of citric acid, we carried out studies to test the potential of fruit wastes like orange peel, sweet lime peel, banana peel and pineapple peel as a substrate to produce citric acid.

MATERIALS AND METHODS

Isolation of fungi

Determination of fungal load from fruit waste dumped soil

Fungal isolates were obtained from the fruit waste dumped soil. The soil samples were collected in sterile polythene bags and brought to the laboratory for further analysis. The soil sample was serially diluted, spread plate method was performed using the dilutions 10^{-3} , 10^{-4} and 10^{-5} in RBA medium and incubated at 30°C for 5 days. After incubation, the black color colonies were suspected as *Aspergillus sp* and selected for the screening of citric acid production.

Screening of fungal isolates for citric acid production

The fungal isolates were subjected to primary screening for the production of citric acid as per the method described by¹². Czapek-Dox (CD) agar medium was prepared with bromocresol green indicator and plated on to the sterile Petri plates. After solidification, 0.5 ml of spore suspension of fungal isolates were transferred to each of the Petri plates and incubated at 30°C for 5 days. Formation of yellow zones around the colony is the indication of citric acid producers. The isolates that produced the widest yellow zone were selected for further studies.

Identification of fungal isolates

The citric acid-producing fungal isolates were subcultured on to PDA plates, incubated at room temperature for five days. After incubation, the isolates were identified based on their cultural characteristics and microscopic observation using Lactophenol Cotton Blue (LPCB) staining method.

Preparation of substrate for citric acid production

Collection and pretreatment of fruit waste samples

The fruit waste samples *viz.*, orange peel, sweet lime peel, banana peel and pineapple peel were collected from local fruit market and juice shop located around Chidambaram Taluk. The collected fruit waste samples were dried in a hot air oven at 60°C for 2 hrs. Then the dried samples were cut into 2 mm sized particles, were used as substrate for citric acid production.

Production of citric acid

Inoculum preparation

The strains of *A. niger* were grown on PDA plates at a temperature of 28°C for 5 to 7 days. After incubation, the plates were washed with sterile 0.1% of Tween 80 solution to collect the spores. The number of spores was counted using haemocytometer. The suspension containing 10^8 spores/ml were citric acid is used as an ingredient for production⁷.

Fermentation

The basic medium for fermentation was prepared by adding 5 g (5%) pretreated substrate in 100 ml of distilled water.

Five grams of each substrate were taken in separate flasks and sterilized at 121°C, 15 lbs pressure for 15 min. Autoclaving of the substrate was done to provide proper cooking of the substrate and to kill the microorganisms. After sterilization, the media were inoculated with strains of *A. niger* (10^8 spores/ml) and incubated at 30°C for 5 days. Methanol 3% (w/v) was added to the medium before inoculation. After fermentation, the media were diluted with the addition of 100 ml of distilled water and then filtered by using Whatman No.1 filter paper. The resulting filtrate was used for subsequent analysis.

Determination of citric acid

Citric acid was determined titrimetrically (AOAC, 1995) by using 0.1 N NaOH and phenolphthalin as an indicator. The percentage of citric acid was calculated according to the following formula:

$$\% \text{ Citric acid} = \frac{\text{Normality} \times \text{Volume of NaOH} \times \text{Equiv. Wt. of citric acid}}{\text{Weight of sample (g)} \times 10}$$

Optimization studies

Parameter optima are important in any type of fermentation process, because the production may get an increase or decrease in respect to media components, pH, temperature, media components, *etc.* To study the effect of above-said parameters, the following experiments were carried out.

i. Effect of medium components on citric acid production

Study the impact of different carbon sources on citric acid, the basal media were supplemented with glucose, sucrose, fructose, and maltose (5 to 15% (w/v)) and the fermentation was carried up to 5 days at 30°C. The culture filtrate obtained after fermentation was analyzed for citric acid production.

ii. Effect of nitrogen sources

The effect of different nitrogen sources on the fermentation was studied by incorporating ammonium phosphate, potassium hydrogen phosphate and peptone (0.5%). The culture filtrate was taken after fermentation was analyzed for citric acid production.

iii. Effect of lower alcohols

The effect of lower alcohol incorporation on the fermentation of citric acid was studied. The media were added with methanol and ethanol at a different concentration ranging from 1 to 4%. The resulting culture filtrate after fermentation was used to estimate citric acid production.

iv. Effect of trace elements

To study the effect of trace elements, the media were introduced with copper, iron and zinc at a concentration of 10 ppm. The culture filtrate was analyzed for citric acid production.

v. Effect of pH.

To study the effect of pH on the fermentation of citric acid, the media were adjusted with different pH ranging from 2 to 6. The culture filtrate obtained after fermentation was analyzed for citric acid production.

vi. Effect of temperature

The influence of temperature on the production of citric acid was determined. The fermentation media were incubated at different temperature levels *viz.*, 20°C, 25°C, 30°C and 35°C.

After fermentation, the culture filtrate of the media was taken to estimate citric acid production.

EXPERIMENTAL RESULTS

The fruit wastes such as orange peel, sweet lime peel, banana peel and pineapple peel were collected, dried and cut into 2 mm sized particles. These particles were used throughout the study.

Determination of fungal load from fruit waste dumped soil

Serial dilution technique was followed to determine the fungal load of fruit waste dumped soil. The fungal load of the samples was determined as 52×10^2 CFU / gm; Black colored colonies were selected for the screening of citric acid production.

Screening of fungal isolates for citric acid production

About 6 isolates were obtained from the fruit waste dumped soil and they were subjected to screening for citric acid production. The isolates were designated as FI -1 to FI- 6 for convenience. Among the 6 isolates, two isolates (FI-1 and FI-3) produced the widest zone and they were selected for further studies (Table.1).

Table-1: Screening of fungal isolates for citric acid production

S. No	Isolates	Citric acid production
1.	FI-1	++++
2.	FI-2	+
3.	FI-3	++++
4.	FI-4	-
5.	FI-5	-
6.	FI-6	+

- Indicates no citric acid production,
+ Least producers of citric acid production,
++++ Best producers of citric acid production.

Citric acid production

The citric acids produced from different fruit wastes were estimated and the results presented in **Figure -1**. Among the four fruit wastes used, citric acid production was maximum from orange peel, i.e. 6.0%, 9.8% by *A. niger* I and *A. niger* II respectively. Then it was followed by sweet lime peel, pineapple peel and banana peel respectively. *A. niger* II gave better yields when compared with *A. niger* I. So, further studies were carried out by employing orange peel as a substrate and *A. niger* II as a fermentative organism.

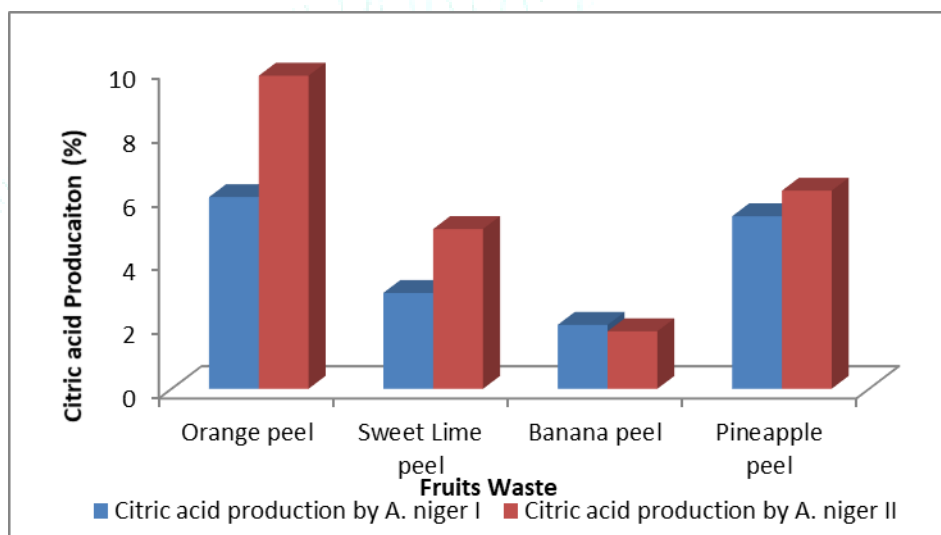


Figure: 1 Citric acid production by *A. niger* strains from different fruit wastes

Optimization of the fermentation process

Effect of pH, Temperature, Trace Elements, Lower Alcohols

Citric acid production by *A. niger* II from orange peels with different concentration of glucose, sucrose, fructose and maltose were analyzed. Among the different carbon sources tested, sucrose supplementation showed better yields of citric acid. After 6 days of incubation, the results indicated that maximum production of citric acid (13.6%) was observed with 10% sucrose supplemented fermentation media. Sucrose concentrations such as 5% and 15% also showed better yield (9.2%, 12.8%) than the other sugars tested.

Table-2 Effect of lower alcohols on citric acid production

S. No	Concentrations used (%)	Citric acid production (%)	
		Methanol	Ethanol
1	1	12.8	13.6
2	2	13.9	12.2
3	3	10.4	9.0
4	4	7.4	7.0

Table-3: Effect of trace elements on citric acid production

S. No	Trace elements (10 ppm)	Citric acid production (%)
1.	Cu ²⁺	13.0
2.	Fe ²⁺	9.0
3.	Zn ²⁺	11.8

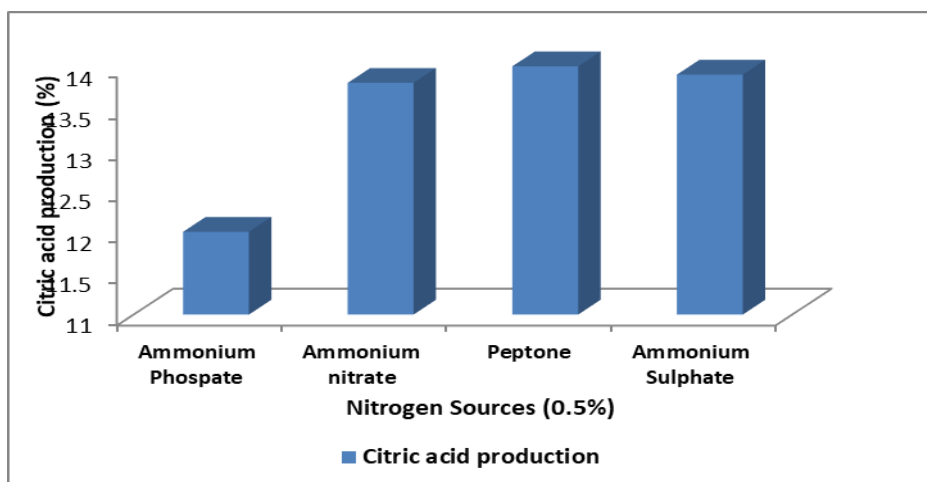


Figure: 2 Effect of nitrogen sources on citric acid production using orange peels as a substrate

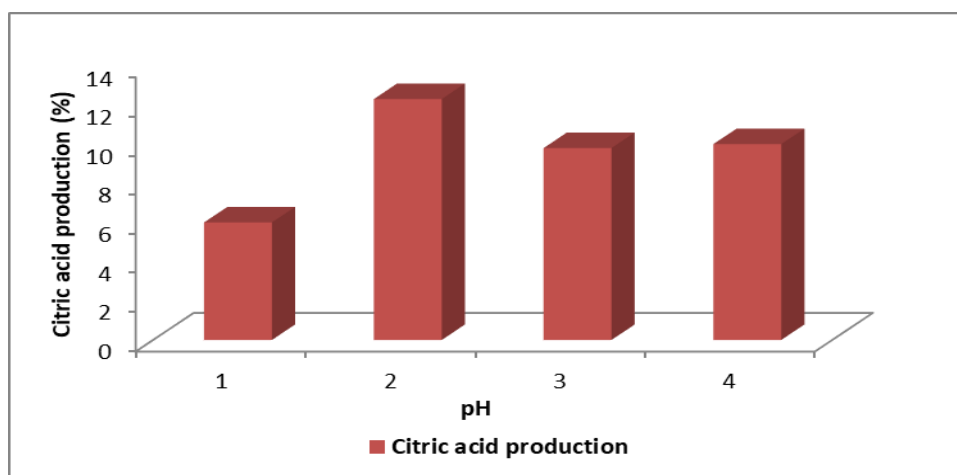


Figure: 3: Effect of pH on citric acid production

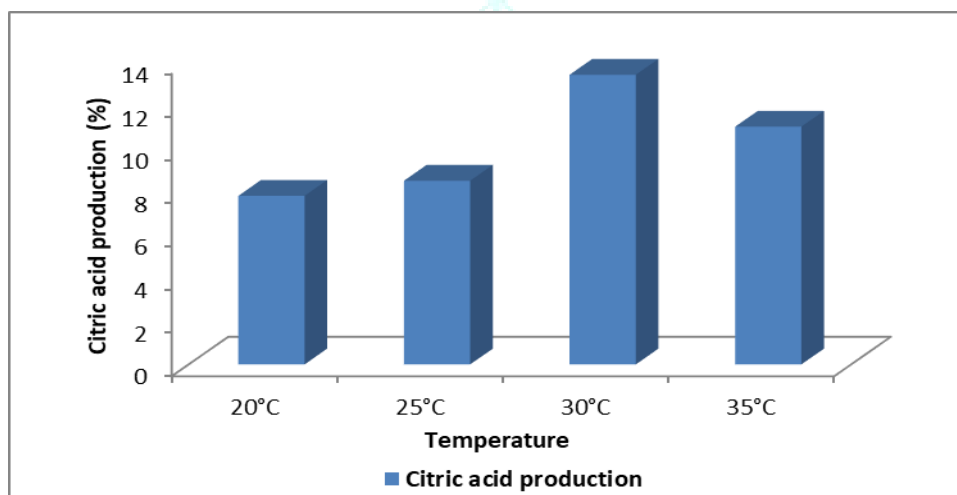


Figure: 4: Effect of temperature on citric acid production

DISCUSSION

Organic acids have a long history of being utilized as food additives and preservatives for preventing food spoilage and extending the shelf life of food¹⁴. The main organic acids in industrial use are citric, acetic, tartaric, malic, lactic and gluconic acid. The most utilized organic acid is citric acid^{15, 16}.

The present investigation was mainly focused on the fruit wastes like orange peel, sweet lime peel, banana peel and

pineapple peel as a potential substrate for the production of citric acid using *A. niger* isolates. Among the six isolates obtained from fruit, waste dumped soil, two isolates were better producers of citric acid (*A. niger* I and II). The best fungal isolates were identified through morphological characteristics by LPCB staining method and colony characteristics on PDA plates.¹⁷ Isolated *A. niger* RCNM17 from the soil of rotten fruit dump outside a wholesale fruit stall.¹⁸ Isolated three fungal isolates, among them only one isolate of *A. niger* produced maximum citric acid from

different agronomic waste viz., grape, orange, apple, vegetable, tapioca and coconut husk. In our study, the isolate *A. niger* II produced maximum amount of citric acid (9.8%) from orange peel. Then it was followed by pineapple peel (6.2%), sweet lime peel (5.0%) and banana peel (1.8%) respectively. (fig-1). Many authors used different variety of fruit wastes for the production of citric acid. They produced citric acid by utilizing different fruit wastes such as grape pomace, orange peel, kiwifruit peel^{19,20}, pineapple wastes, citrus waste and banana peel^{21,22}.¹²Used different varieties of banana peel for producing citric acid by *A. niger*.

Parameter optima are of considerable importance to increase citric acid yield. The best isolate *A. niger* II and orange peels alone selected for optimization studies. Citric acid production from orange peel was tested at different pH levels in order to optimize the pH. pH did not have any influence within the range of 1.95 to 3.10²³.²⁴Have indicated that a pH of 2.5 is a clear optimum for final product concentration. Herein, pH 3.0 was found to be optimum for better production of citric acid (12.3%). Likewise,²⁵recommended that pH should be kept low, for the better yield of citric acid (Fig.3).

Effect of temperature on the citric acid production by the different organisms was submitted by many authors.²⁶Stated that incubation temperature should be in the range of 28 to 32°C. In our study, the temperature of 30°C was better for citric acid production (13.4%). If the temperature decreases or increases above 30°C, the production rate also gets reduced. Similar results were observed with the study conducted by¹² (Fig.4).

²⁷Studied the impact of different types and concentration of sugar on citric acid production. Herein, different carbon sources were tried, among them sucrose (10%) yielded the maximum amount of citric acid (13.6%), followed by glucose, maltose and then fructose. This result is agreed with¹², they proposed that citric acid yield was maximum from pineapple waste when sucrose (15%) used as a carbon source.²⁸Stated that sucrose can be readily attacked by the microbial intracellular enzymes, because of its low molecular weight.²⁹Found that nutrient medium incorporated with sucrose recorded maximum citric acid production than the glucose added medium. They concluded that glucose is not suitable carbon source for higher production of citric acid by *A.niger*.

The nitrogen source of the fermentation medium will have a direct effect on the yield of citric acid³⁰. Effect of different nitrogen sources on citric acid production by *A. niger* was studied by³¹. They found that the optimal concentration of ammonium sulphate was 5 kg m⁻³.³²Reported that citric acid production starts only after the consumption of nitrogen sources. In our investigation, 4 different nitrogen sources were used. Among the four, peptone (0.5%) yielded maximum citric acid (14.6%) followed by ammonium sulphate, ammonium nitrate and ammonium phosphate respectively (Fig-2). Likewise,¹² reported that supplementation of the basal medium with ammonium nitrate (0.25%) gave increase in citric acid production from pineapple waste.³³Stated that ammonium sulphate incorporation increased the yield of citric acid from tapioca and pineapple waste.

The addition of lower alcohols like methanol, ethanol, n-propanol and carbohydrate raw materials could increase the yield of citric acid²³.³⁴Stated that alcohol could be used as a stimulant to enhance the yield of citric acid. According to³⁵ the stimulatory effect of methanol may be due to reduction in the inhibitory effects of metal ions. In the present work, effect of methanol and ethanol on citric acid production

were compared. Methanol at 2% concentration gave 13.9% of citric acid whereas ethanol 1% recorded 13.6% of citric acid. (Table-2). These results are comparable with the results obtained by^{12,36}. As per them, lower concentration of methanol has stimulatory impact on citric acid production.³⁷Reported that both methanol and ethanol under optimized conditions increases the productivity of citric acid.

Trace element supplementation in the fermentation media plays a role in affecting the yields of citric acid. The trace elements addition should be optimum unless they will not allow high production. As indicated by Yogitoglu (1992)²⁴, the level of manganese, iron, copper and zinc are quite critical. If the levels of these trace elements are other factors have less pronounced effects. In our study, three trace elements viz., Cu²⁺, Fe²⁺ and Zn²⁺ were incorporated to study their effect on citric acid production. Higher yields of citric acid (13.0%) observed with Cu²⁺ added fermentation media prepared with orange peels (Table-3).³⁶Reported that copper and zinc favored citric acid production while iron and manganese displayed harmful impact on the bioaccumulation of citric acid.

CONCLUSION

Based on the results of the present study, it could be concluded that the fruit waste *i.e.* orange peel is a potential substrate to produce citric acid. The study explored the efficient way to minimize environmental pollution due to dumping of fruit wastes. Citric acid production by utilizing the fruit waste as the substrate is very economical, especially when the fermentation conditions are optimized. Supplementation of nutrients on to the media had high degree of positive correlation to the yield of citric acid. This study also represents that the production of citric acid from fruit waste is an efficient way of recycling the waste generated from fruit processing industries.

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