# Effect of physical parameters on Citric Acid production by *Aspergillus -niger ARU1 LC 541742* from Fruit Waste.

**R. K. Kamble* D. U. Gawai**

Department of Botany, Science College Nanded, Nanded-415306 (MS), India.

***ABSTRACT:*** Citric acid is one of the most significant organic acids having an ever ending demand in the world. The two most principal microbial sources are fungi and bacteria. But fungus remains the ideal sources for the production of citric acid. *Aspergillus niger* was the most commonly used fungus for the citric acid production due to its high yield and relatively high tolerance to the acid accumulation (Pandey P., 2013). It has been employed by many researchers and in many research studies, chiefly in solid-state fermentation (SSF), for its ability to live and grow in an environment analogous to its natural habitat. In the last 3 decades, solid-state fermentation (SSF) has gained great interest from researchers and industries as a complementary technique to the traditionally used submerged fermentation (SMF). The unique characteristics of solid-state fermentation, using solid materials, stimulated researchers to utilize the waste such as agro residual and agro industrial wastes, fruit waste as an substitute to raw materials for the citric acid production. Several advantages of Solid state fermentation over submerged fermentation have encouraged researchers to study and expand it, such as less risk of bacterial contamination, lower energy prerequisites and less environmental concerns regarding the disposal of solid waste (Sadia Javed, 2011).

***Keywords****: Citric acid; Aspergillus niger;* Orange Fruit Peel

## Introduction:

One of the very important fungi used in fermentation industrial, *A. niger* has been employed for economical production of citric acid (Schuster *et al*., 2002). According to Lesniak *et al*., 2002, bulk hydrated materials and sugar by-product for production by *A. niger* the citric acid is produced commercially from the fermentation. According to Alvarez-Vasquez *et al*., 2000, the requirement of worldwide more economical processes are

required for citric acid. It was distinguished fact that the growth and production of *Aspergillus niger* are affected by the fermentation parameters and stimulators, medium composition.In the present study fruit peels of Orange Fruit Peels are collected from fruit vendors for the citric acid production by using *Aspergillus niger*. This displays an alternative source for the waste management of fruit waste and extracting best out of waste.

## MATERIALS AND METHODS

### Collection of substrates

Orange Fruit Peels were collected from fruit vendors.

**Pretreatment of fruit peels**: The collected peels were oven dried at $60^0$c for 2hours for dry fruit peels and used for production of citric acid.

**Fermentation: Solid-substrate fermentation is carried out to produce citric acid**

**i. Preparation of fermentation media:**

The basal medium was prepared by introducing fresh peels Orange Fruit Peels of (30 to 60 gms) into separate 100ml Erlenmeyer flask. (Kareem S.O. *et al.*, 2010)

**13. Study of effect of physical parameters on citric acid production**

**i. Effect of temperature**

About 30g of the fruit substrates were taken in 250ml Erlenmeyer flasks and prepared for solid state fermentation as described earlier. Thus, prepared flasks were inoculated at different temperatures like $20^0$C, $25^0$C, $30^0$C, $35^0$C and $40^0$C. The results were recorded.

**ii. Effect of incubation time**

Citric acid production was recorded at different incubation time for different fruit peels. Citric acid recorded at 5.0, 7.0, 9.0 incubation days showed decline after day 10.The decrease in citric acid production can be accounted to the decrease in sugar content and the growth phase of fungi.

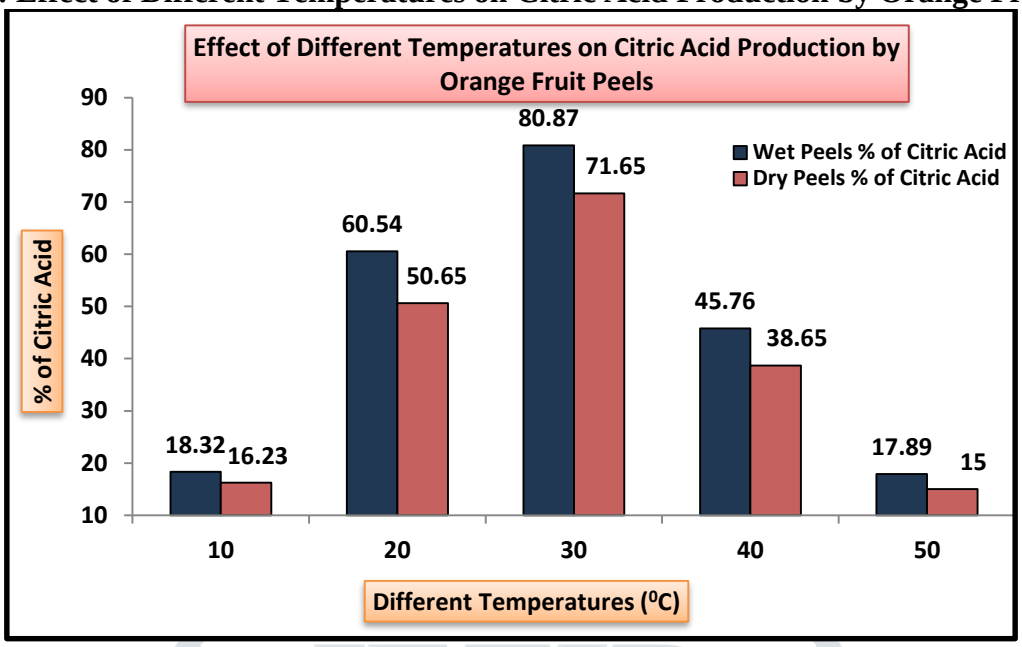
## RESULTS AND DISCUSSION

**1. Effect of temperature**

**Table 1: Effect of Different Temperatures on Citric Acid Production by Orange Fruit Peels**

| Sr. No. | Fermentation Substrate | Temperature ($^0$C) | Titrable acidity | |
|---|---|---|---|---|
| | | | Wet Peels (% citric acid) | Dry Peels (% citric acid) |
| 1. | Orange | $10^0$C | 18.32 | 16.23 |
| | | $20^0$C | 60.54 | 50.65 |
| 2. | | $30^0$C | 80.87 | 71.65 |
| 3. | | $40^0$C | 45.76 | 38.65 |
| | | $50^{0C}$ | 17.89 | 15.00 |

**Figure 1: Effect of Different Temperatures on Citric Acid Production by Orange Fruit Peels**



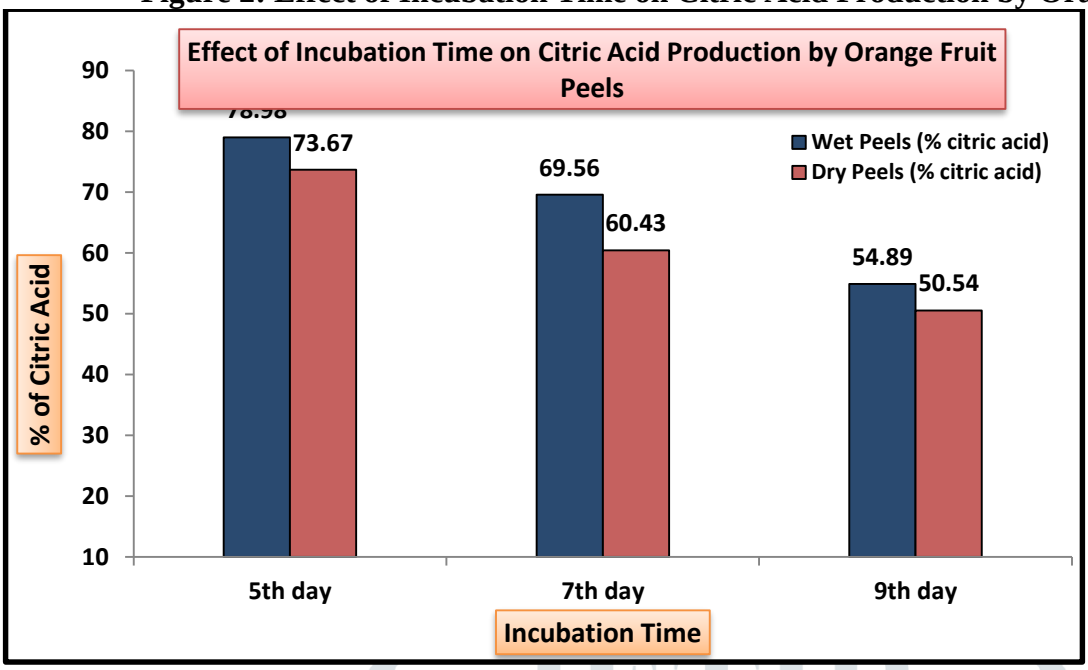

It evident from the **Figure 1** that the amount of citric acid production by the given isolate at different temperatures such as $10^0$C, $20^0$C, $30^0$C, $40^0$C and $50^0$C using fresh wet peels was up 18.32%, 60.54%, 80.87%, 45.76% % and 17.89% respectively whereas 16.23%, 50.65%, 71.65%, 38.65% and 15.00% in dry peels of orange when incubated at rotary shaker operating at 100rpm for 72 hours.From the results it is clear that among the various temperatures tested; maximum citric acid production by *Aspergillus niger ARU1* using solid state fermentation (SSF) was recorded at $30^0$C. On the other hand, least amount of citric acid production was recorded at $50^0$C (**Figure 1**). Therefore, the optimum temperature for maximum citric acid production using fresh wet and dry orange fruit peels was found to be $30^0$C.In a low or high incubation temperature, citric acid synthesis could be affected by a retarded germination of fungi, slow metabolic activity, enzyme denaturation and low cell viability.

**ii. Effect of incubation time**

**Table No. 2 Effect of Incubation Time on Citric Acid Production by Orange Fruit Peels**

| Sr. No. | Fermentation Substrate | Amount of Substrate taken | Incubation Time | Titrable acidity | |
|---|---|---|---|---|---|
| | | | | Wet Peels (% citric acid) | Dry Peels (% citric acid) |
| 1. | Orange | 30g | $5^{th}$day | 78.98 | 73.67 |
| 2. | | | $7^{th}$day | 69.56 | 60.43 |
| 3. | | | $9^{th}$ day | 54.89 | 50.54 |

**Figure 2: Effect of Incubation Time on Citric Acid Production by Orange Fruit Peels**




It evident from the **Figure 2** that the amount of citric acid production by the given isolate at different incubation time such as 5th day, 7th day and 9th day using fresh wet peels was up 78.98%, 69.56% and 54.89% respectively whereas 73.67%, 60.43% and 50.54% respectively in dry peels of orange when incubated at rotary shaker operating at 100rpm.

In the present investigation, the rate of citric acid biosynthesis was studied and the maximum citric acid i.e. 78.98% in wet peels and 73.67% in dry peels was achieved 5th day after inoculation by the strain *Aspergillus niger ARU1*. Considering the incubation period, in this study the Citric acid amount increased and assayed high value among 5th day incubation, although the fungal growth started reduces at 7th day incubation.From the results it is clear that among the various incubation time tested; maximum citric acid production by *Aspergillus niger ARU1* using solid state fermentation (SSF) was recorded at 5th day of incubation. On the other hand, less amount of citric acid production was recorded at 9th day (**Figure 2**). Therefore, the optimum incubation time for maximum citric acid production using fresh wet and dry orange fruit peels was found to be 5th day of incubation.

## CONCLUSION

The optimal time of incubation for maximum citric acid production varies with both the organism and fermentation conditions. The rate of citric acid biosynthesis was studied and the maximum yield of citric acid was produced after 5 days of fermentation. Extension of the fermentation period brought about depletion in the yield of citric acid produced. In batch-wise fermentation of citric acid, the production started after a lag phase of one day and reached maximum at the onset of stationary phase or later. It might be due to the decreased available nitrogen in fermentation medium, the age of fungi, and depletion of sugar contents. Similar type of work has also been reported by Wieczorek and Brauer (1998). When the temperature of medium was low, the enzyme activity was also low, which gives no impact on the citric acid production. But when the temperature of

medium was increased above $30^0$C, the biosynthesis of citric acid decreased. It might be due to the accumulation of different by-products such as oxalic acid. Similar results were also obtained by various workers by using $30^0$C as the cultivation temperature and obtained higher production of citric acid (Vergano *et al*., 1996; Arzumanov *et al*., 2000).

**References**


1. Hossain, M., Brooks, J. D. and Moddax, I. S. (1984).The effect of the sugar source oncitric acid production by Aspergillusniger. Applied Microbiology and Biotechnology 19,393-397.
2. Hildegard K., Rumia, G., and Yigal, H. 1981.Citric acid fermentation by Aspergillusniger on lowsugar concentrations andcotton waste. Appl. Environ. Microbiol.42: 1-4.
3. Rouskas, T., and Kotzekidou, P. 1997.Pretreatment of date syrup to increase citric acidproduction. Enzyme. Microb. Technol. 4(21): 273-276.
4. Hayder Khaleel Qurban Al1i, Economic benefit from the optimization of citric acidproduction from rice straw through Plackett-Burman design and central compositedesign, Turkish J. Eng. Env. Sci. 36, 81 – 93, 2012.
5. Omoniyi Kuforiji, Orange and pineapple wastes as potential substrates for citric acidproduction, International Journal of Plant Biology 2Vol 1, No 1; 1-4, 2010.
6. Pandey. Petal, Studies on citric acid production by Aspergillusniger in batchfermentation, Recent Research in Science and Technology, 5(2): 66-67, 2013.
7. SadiaJaved, Enhanced citric acid production by AspergillusnigerEB-3 mutant using aninert solid support in molasses Medium, African Journal of Biotechnology Vol. 10(55),pp. 11784-11791, 21 September, 2011.
8. Cappuccino Sherman, fungal identification, A laboratory manual, Pearson education, 6thedition, 122.
9. Kareem S.O. and Rahman R.A, Utilization of banana peels for citric acid production byAspergillusniger, Agriculture and Biology Journal of North America, 4(4): 384-387, 2011.
10. Kareem S. O., Production of citric acid by Aspergillusniger using pineapple waste,Malaysian Journal of Microbiology, Vol 6(2), 161-165, 2010.
11. Dhandayuthapani. K, Production of citric acid from banana waste by Aspergillus niger,International Journal on Applied Bioengineering, Vol.2, No.1, July 2008
12. Rashad R. Al-Hindi, Ahmed R. Al-Najada and Saleh A. Mohamed. Full Length Research Paper Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes African Journal of Microbiology Research Vol. 5(4), pp. 443-448, 18 February, 2011 Available online http://www.academicjournals.org/ajmr DOI: 10.5897/AJMR10.896 ISSN 1996-0808