ECONOMICAL PRODUCTION OF CITRIC ACID FROM VEGETABLE WASTE

Naaz Abbas, Nimra Shahbaz,Warda Sharif, Sania Mazhar, Zainab Abbas and Sakhawat Ali
PCSIR Laboratories Complex, Ferozepur Road, Lahore-54600, Pakistan
Corresponding Email: drnaazabbas@gmail.com

ABSTRACT: *Pertaining to essential industry wide usage citric acid is produced through several methods. This paper led to investigate the production of citric acid by using dehydrated and non-dehydrated vegetative waste as a substrate by Aspergillus niger through submerged fermentation. During several stages of the production, it was witnessed that maximum production of citric acid was attained by the sweet potato with glucose 50g/l and potato with fructose 42.24g/l in fermented broth at 30^{o}C for 11 days. The partial recovery of the citric acid was accomplished by the crystallization and estimated by high performance liquid chromatography.* 6.18g/L crystals of citric acid were recovered.

Key Words: Citric acid, submerged fermentation, Aspergillus niger, Sweet potato peels, potato peels

1. INTRODUCTION

Citric acid is a weak organic acid that is found in Citrus fruits including oranges, lemons, grape fruits, limes and pineapples [1]. Citric acid is also produced during the TCA (Kerb's) cycle and so, the part of metabolism of almost all the aerobic organisms **[**2**]**. It helps in maintaining energy levels and detoxification, also helps in proper functioning of kidney and healthy digestion [1]. Citric acid has applications in food, pharmaceutical, medical and chemical industries. 70% of the total citric acid production used in the food industry and the remaining 30% is used in other industries including pharmaceutical, medical and chemical industries [3]. Citric acid has the vast use in food and beverage industries for the preservation of food because of its antioxidant properties. It is used for balancing the sweetness of juices, soft drinks and beverages [1].

Citric acid fermentation can be carried out by the number of microorganisms such as fungi, yeast and bacteria. *Arthrobacter paraffinens, Bacillus licheniformi*s and *Corynebacterium sp* are the bacterial species that have the potential to produce citric acid. Yeast species includes *Candida tropical is* and *Hansenula anomala* which are employed for citric acid production whereas fungal species are considered as more important because of ease in production and better production results. Among them *Penicillium janthinellum* and *Aspergillus awamori*can be used for citric acid production but the most important one is *Aspergillus niger* [4].

Demand for citric acid is increases day by day due to its vast applications. Constant increase in consumption of citric acid is noted to be 3.5-4% each year which also increase the price of citric acid. [5]. Therefore, it is necessary to find the cheap methods for citric acid production. It is estimated that about 70 to 140 thousand tons of potato peels as a waste material are produced from industries each year [6] and about 7% of the total global amount of sweet potato go to waste according to the FAO food balance sheets 2011. Several reports showed production of citric acid using fruit peels [7][8]. The present study outlines the production of citric acid by the use of dehydrated and non-dehydrated vegetative waste by fermentation using *Aspergillus niger* and optimization of fermentation parameters. Objective of this study was to determine cheap substrates for the production of citric acid for combating the increasing prices of citric acid in the market and the removal of waste from the environment.

2. MATERIALS AND METHODS

Whole research work was carried out at Biotechnology section at PCSIR Laboratories Complex, Lahore, Pakistan.

2.1. Inoculum preparation

Aspergillus niger strain was obtained from Molecular Biology laboratory of LLC and incubated on PDA agar plate at 30^{o}C for 5 days. Inoculum was prepared by washing the spores of Aspergillus niger with 10 ml autoclaved distilled water. The spore suspension prepared was used as the inoculum for fermentation media.

2.2. Substrate media preparation

Potato, Sweet potato, Turnip and Radish were purchased from the local market of Muridke Punjab, Pakistan and washed with the clean tap water to remove dirt and peeled manually by the peeler, sun dried for 5 days and grinded for making fine powder. Substrate media was prepared by adding 2.5g of Potato and Sweet potato peels powder or paste (forms in a mortar and pestle) individually in 50 ml of distilled water. Media was autoclaved at 121^{o}C for 45 minutes and then cooled to room temperature. Control media was also prepared with same concentration.

2.3. Fermentation method

Submerged fermentation method was adopted for this study. The fermentation media was inoculated with 1% inoculum aseptically. Media was stirred and incubated for 11 days at 30^{o}C in the incubator. Then the production of citric acid was estimated by titration.

2.4. Assay

Procedure for calculation of Citric acid

Fermentation media was taken out from incubator and filtered with Whatman filter paper no. 1 in a 250 ml Erlenmeyer flask. Then the filtrate was diluted up to 5% with distilled water and 2-3 drops of 0.1% Phenolphthalein was added as an indicator. 0.1N NaOH was added drop wise to the filtrate by burette until the pink color appeared which indicated the end point of the titration. Then the following formula was used for calculating the citric acid percentage.

$$\%CA = \frac{Normality\ X\ Volume\ of\ NaOH\ X\ Equiv.wt\ of\ Citric\ acid}{Weight\ of\ sample\ (g) X\ 1000} X100$$

2.5. Optimization of Parameters

The parameters for fermentation of citric acid from hydrated and dehydrated vegetative waste by using Aspergillus niger were optimized. The parameters monitored were include Incubation time, Types of Substrate, Substrates concentration, effect of Carbohydrates and shaking during

fermentation. Only one Parameter at a time is monitored and the others kept constant.

2.5.1. Effect of Incubation time

Citric acid production was studied by incubating fermentation media for different days. They were 5, 8 and 11 days by incubating the fermentation media by *Aspergillus niger* individually. .

2.5.2. Effect of Type of Substrates

For checking the effect of type of substrate on the production of citric acid by *Aspergillus niger*, four types of substrates (Radish, Turnip, Potato and Sweet potato hydrated and dehydrated peels) were used. Production of citric acid with Radish, Turnip, Potato and Sweet potato peels by *Aspergillus niger* is carried out individually at 30°C for 11 days.

2.5.3. Effect of Substrate Concentration

The effect of substrate concentration on citric acid production by *Aspergillus niger* was determined by individually incubating the fermentation media with different concentrations (1%, 2%, 3% and 5%).The citric acid determination was carried out after 11 days at 30°C.

2.5.4. Effect of Carbohydrates

The effect of Carbohydrates on citric acid production by *Aspergillus niger* was determined by incubating the Potato and Sweet potato substrate with different types of carbohydrates (2%Glucose, 2%Lactose, 2% Starch, 2%Fructose and 2%Maltose) individually at 30°C for 11 days.

2.5.5. Effect of Shaking

Effect of shaking on citric acid production was monitored by incubating the fermentation media with all parameters in a shaking incubator for 11 days at 30°C. A parallel batch was incubated without shaking.

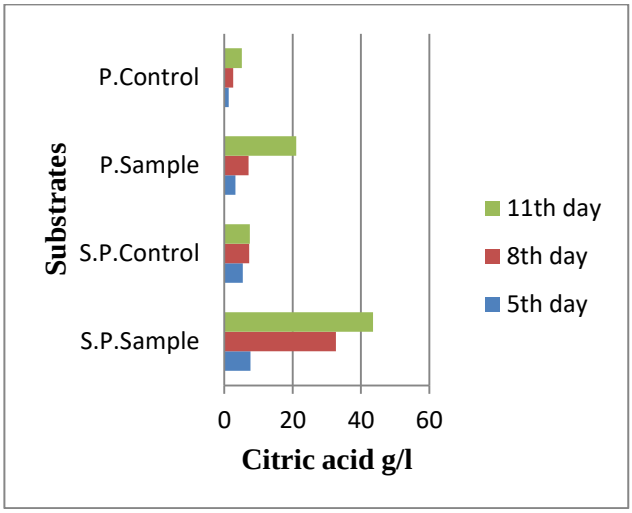
2.5.6. Product recovery

After fermentation, fermented media was filtered with Whatmann filter paper no.1 and the filtrate was used for crystallization of citric acid. For precipitation of citric acid, pH of the fermented broth was increased from 3 to 9 by using 10% NaOH solution. Treated solution was filtered by filter paper and transferred to the beaker. Then calcium chloride was added to form calcium citrate, mixed and heated the solution at 100°C for 7 minutes for obtaining precipitate. Obtained precipitate was filtered and transferred to the beaker. Some amount of sulfuric acid was added to the precipitate and mixed. Then the solution was filtered for separating calcium sulfate and dissolved in distilled water. Water was evaporated from solution for recovery of citric acid crystals [7].

3. RESULTS

3.1. Effect of Incubation time

Citric acid production was studied at different incubation days**.** They were 5, 8 and 11 days by incubating the fermentation media by *Aspergillus niger* individually. Maximum production of citric acid was noted at 11th day of incubation. After 11 days, decrease in citric acid values was noted.



Figuer1: Illustration of citric acid production from dehydrated sweet potato (SP) and potato (P) peels fermentation using *A. niger* at different incubation time

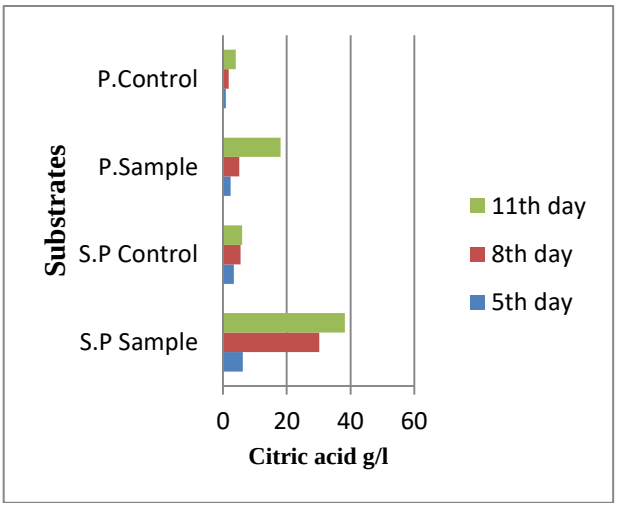


Figure 2: Illustration of citric acid production from non-dehydrated sweet potato and potato peels Fermentation using *A.Niger* at different incubation time

3.2. Effect of Type of Substrates

Four types of substrates were used for checking the effect of type of substrate on the production of citric acid by *Aspergillus niger* at30°C for 11 days. All the substrates were individually inoculated with *Aspergillus niger* and incubated at 30°C for 11 days. The substrates used were radish, turnip, potato and sweet potato. Maximum production of citric acid was noted with sweet potato dehydrated peels and the low production of citric acid was noted with radish and turnip dehydrated peels.

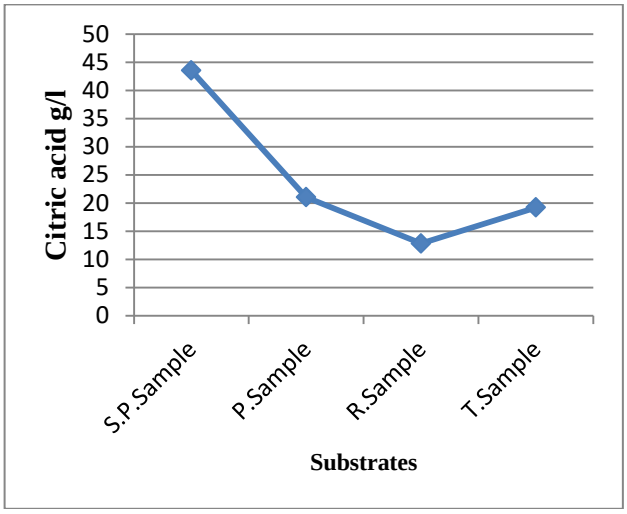


Figure 3: Illustration of citric acid production from non-dehydrated sweet potato (SP), potato (P), Turnip (T) and Radish (R) peels fermentation using *A. niger* at the 11th day of incubation.

3.3. Effect of Substrate Concentration

Substrate media with 1%, 2%, 3% and 5% concentrations were prepared for checking the effect of substrate concentration on citric acid production.5% substrate concentration was found to be more effective for the production of citric acid. Increase in substrate concentration from 5% causes the decrease in citric acid production due to the increase in fermentation media viscosity.

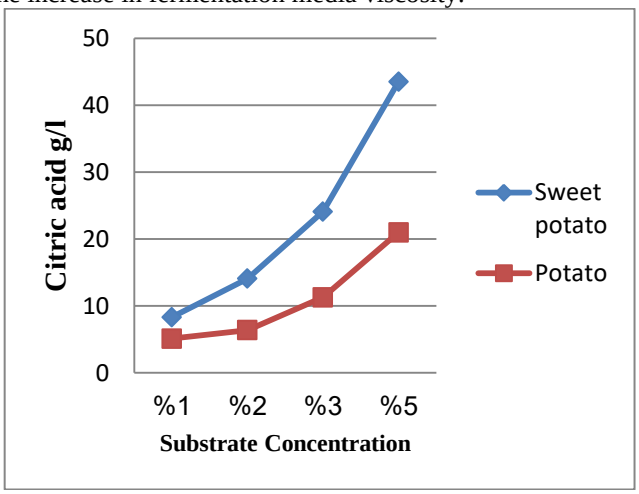


Figure 4: Illustration of citric acid production from fermentation of different concentrations of substrate Using *A. niger*

3.4. Effect of Carbohydrates

The effect of Carbohydrates on citric acid production by *Aspergillus niger* was determined by incubating the Potato and Sweet potato substrate with different types of carbohydrates (2%Glucose, 2%Lactose, 2%Starch, 2%Fructose and 2%Maltose) individually at 30°C for 11 days. Maximum production of citric acid was found with glucose (50g/l) and fructose (42.24g/l) from sweet potato and potato dehydrated peels respectively. Sweet potato and potato exhibited maximum production of citric acid with glucose and fructose respectively at 11th day. While lactose, Maltose and Fructose shows a different behavior with sweet potato by giving the maximum production of citric acid at the 5th day. Potato dehydrated peels as a substrate also gave the maximum production of citric acid with glucose and maltose at 5th day but with starch at 11th day.

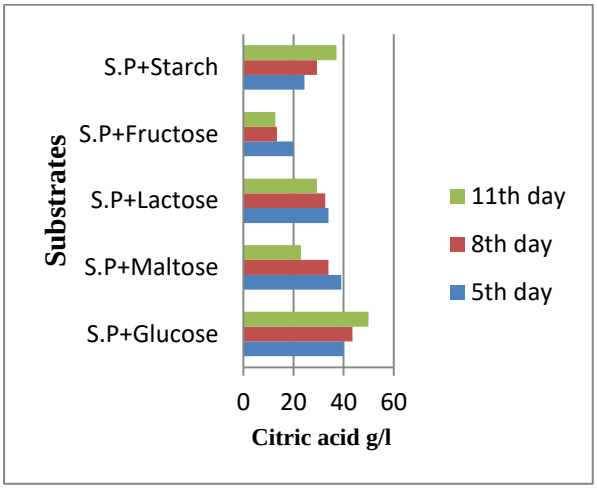


Figure5: Citric acid production from dehydrated sweet potato peels fermentation using *A. niger* with carbohydrates

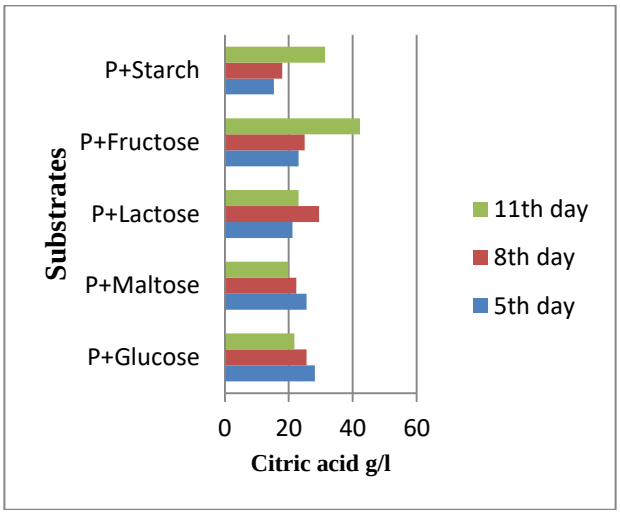


Figure 6: Illustration of citric acid production from dehydrated potato peels fermentation using *A. niger* with carbohydrates

3.5. Effect of Shaking

Effect of shaking on citric acid production was monitored by incubating the fermentation media with all parameters in a shaking incubator for 11 days at 30°C. No effect was monitored on citric acid production by shaking the fermentation media in the shaking incubator.

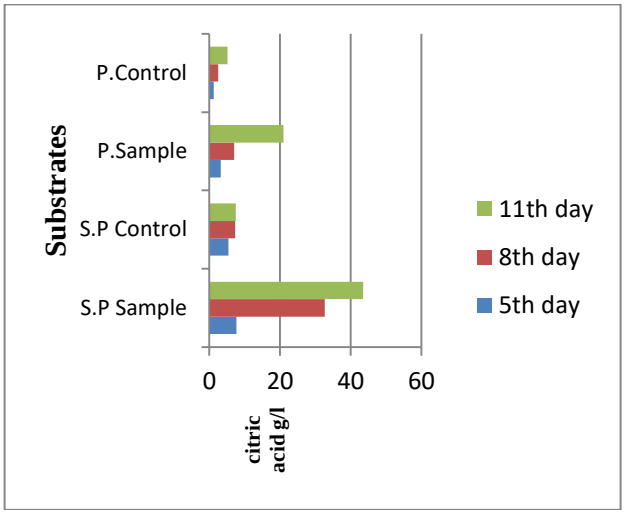


Figure 7: Illustration of citric acid production in shaking incubator from dehydrated sweet potato and potato peels fermentation using *A. niger*

From fermented dehydrated sweet potato peel media, 6.18g/L crystals were recovered (Fig. 8).

Figure 8: Citric Acid recovered from fermented dehydrated sweet potato peel

DISCUSSION

Citric acid gains the importance at commercial level due to its applications in different industries. Now researchers focus on the production of citric acid from cheap substrates that would decrease the cost of citric acid. Utilization of vegetable waste for citric acid production can remove the waste from environment with the production of industrially valuable organic acid through fermentation [9].

Dehydrated and non-dehydrated peels of Radish, Turnip, otato and Sweet potato was used as a substrate for fermentation and each give a different result for citric acid production and depends on the chemical attributes of carbon source. Citric acid production from fermentation of dehydrated and non-dehydrated used Substrates was increased gradually from day 5 to day 11. On the 11th day maximum production of citric acid was noted which coincides with the results of previous studies [10] [11].Citric acid was increased with increase in production period and after it decrease in citric acid production was noted. Decrease in production of citric acid may be due to the insufficient supply of nitrogen in the media, increased age of fungi, depletion of sugar content, inhibition caused by high concentration of produced citric acid and decline of enzyme system which take part in biosynthesis of citric acid [12]. Some amount of citric acid was also noted in the control media.

Addition of different types of carbohydrates in substrate media gives the different results because of the nature of sugar source which has a noticeable effect on the production of citric acid by use of *A. niger* [13] [14]. Dehydrated sweet potato and potato showed maximum production of citric acid with glucose (50g/l) and fructose (42.24g/l) respectively at 11th day. While lactose, Maltose and Fructose showed a different behavior with sweet potato by giving the maximum production of citric acid at the 5th day. Potato dehydrated peels as a substrate also gave the maximum production of citric acid with glucose and maltose at 5th day but with starch at 11th day.

Substrate concentration of 5% was found to be best for all the dehydrated peels. Increase in substrate concentration causes decrease in citric acid production due to the increased viscosity of the media may relate to [15].

CONCLUSION

Citric acid was produced successfully from potato and sweet potato peels by consuming *Aspergillus niger* at 30^{o}C for 11 days using submerged fermentation. It is concluded that use of carbohydrates increases the amount of citric acid produced particularly glucose in sweet potato fermentation and fructose in potato fermentation. Use of glucose in sweet potato fermentation increases the amount of citric acid from 43.52g/l to 50g/l and fructose increases the amount of citric acid in potato fermentation from 21g/l to 42.24g/l. In this method, all the chemicals used can be found at low prices in the local market and also this method is easy to perform with small number of equipment's.

REFERENCES

[1] Bikash, C.B., Rashmiranjan , M., Sonali . Z,"Microbial citric acid: Production, properties, application, and future perspectives",*Food Frontiers*, **2**,.62–76(2021).

[2] Vidya,P., Annapoorani,AM.,Jalalugeen,H"Optimization and utilization of various fruit peel as substrate for citric acid production by AspergillusNiger isolated from orange and carrot", *The Pharma Innovation,***7**,141-146(2018).

[3]Omar,S.,Sagar,M.,Vanitha,R.,Geetha,N.,Ashok, B.,"Fungal Citric Acid Production Using Waste Materials: A Mini-Review",J *Microbial Biotech Food Sci*, **.8**, 821-828(2018).

[4]Sharad,G.,"Comparative Analysis Of Citric Acid Production By AspergillusNiger Using Different Media",*Plant Cell Biotechnology and Molecular Biology*,**22**, 77-85(2021).

[5]Thiruvengadam.S.,Thangavel.S,"Optimization of Citric Acid Production Using Aspergillusniger Isolated from the Leaf Litter Soil of Sathuragiri Hills", *Universal Journal of Microbiology Research*, **4**, 79-87(2016).

[6]M. D. Makut,I. K. Ekeleme,"Citric Acid Production by Aspergillus niger and Trichoderma viride Using

Hydrolysed Potato Peels Substrate",*Makut and* Ekeleme; AJAAR, **5**, 1-7(2018).

[7] Abbas N, Safdar W, Ali S, Choudry S, Elahi S. "Citric Acid Production From Aspergillusniger Using Banana Peel", *IJSER*, **7(1),** 1580-1583(2016)

[8] Abbas N, Safdar W, Ali S, Choudry S, Elahi S, "Citric Acid Production From *Aspergillusniger* Using Mango (*Mangiferaindica L.*) and Sweet Orange (*Citrus sinensis*) Peels as Substrate", *IJSER*, **7(2)**, 868-872 (2016).

[9] Laboni,M.,, Ibrahim. K., Muhammad, K,M., Muhammad, K,A., HO,R., R. Begum., Nadia. A., "Citric acid production by Aspergillus niger using molasses and pumpkin as substrates," *European J. of Biol. Sci.*,**2,**01-08(2010).

[10] Abdullah-AlMahin, A.B.M.Sharifuzzaman, M.O. Faruk, M.A.Kader, J.Alam,R.Begum, Harun-Or-Rashid, " Improved Citric Acid Production by Radiation Mutant Aspergillusniger Using Sugarcane Bagasse Extract",*Biotechnology*,**11**,44-49(2012).

[11] Marlinda, Mardhiyah N, M. Irwan, Ramli,"Citric Acid Production From Molasses Use Biosynthesis Aspergillusniger*",International Journal Of Scientific & Technology Research*, **8**, 357-360(2019).

[12] Ali , S. R. , Anwar , Z. , Irshad , M. , Mukhtar , S. , Warraich , N. T. ,." Bio - synthesis of citric acid from single and co - culture - based fermentation technology using agro – wastes". *J. Rad. Res. Appl. Sci***. 9 (1)** , 57 – 62(2016) .

[13] M.Hossain, J.D.Brooks, I.S.Maddox,"Galactose inhibition of citric acid production from glucose by Aspergillus niger", *Appl. Microbiol. Biotechnol*, .**22**, 98-102(1985)

[14] Ahsan,J., Awais,A., Ali,T., Umair,S., Muhammad,N., Adeela, H,"Potato peel waste—its nutraceutical, industrial and biotechnological applacations", *AIMS Agriculture and Food*, **4**, .807–823(2019).

[15] Winifred,A., Margaret,M,B., Ruben,O.,M,"Potential use of byproducts from cultivation and processing of sweet potatoes", *Ciência Rural*, *Santa Maria*, **47(5)**. *e20160610* (2017).