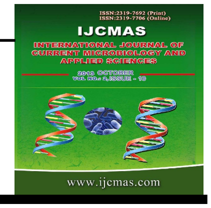

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 10 (2013) pp. 347-352**
http://www.ijcmas.com

Original Research Article

Production and Optimization of Citric Acid by *Aspergillus niger* using Fruit Pulp Waste

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A B S T R A C T

Keywords

Citric acid; *Aspergillus niger;* fruit pulp waste.

Citric acid is a weak organic acid, which is used in many pharmaceutical and in other industrial food products. Increased demand of citric acid has led to search for high yielding fermentable strains of microorganisms and cheaper fermentation substrate in many countries. The present study suggests that in near future waste fruit pulp could be one of the substrate for citric acid production at industrial scale worldwide. Production of citric acid was carried out by *Aspergillus niger* in the medium without methanolas well as withmethanol. Maximum citric acid production was obtained by using pineapple pulp waste i.e., 3.25 g/kg of citric acid in the medium without methanol and5.25g/kg with methanol. Citric acid production was found to be2.75, 2.63, 1.75, and 1.5and 0.75g/kg of the substrate in the medium without methanolwhile, 0.16, 0.13, 0.19, 0.15 and 0.22g/kg of the substrate with methanol by using dry fruit pulp of Indian jujube, Beet root apple, Guava,Papayaand Wood apple respectively.

Introduction

Citric acid is a weak organic acid found in citrus fruit. It is a good, natural preservative and is also used to add an acidic (sour) taste to food and soft drinks. In biochemistry it is important as an intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all form of life. Sometimes a high concentration of citric acid can damage hair, bleach it and can cause skin and eye irritation. The excess citric acid is metabolized and eliminated from the body. It also serves as an environmentally benign cleaning agent and acts as an antioxidant (Hildegard *et al*., 1981; Aysegul and Christian,2003). Chemically, citric acid shares the properties of other carboxylic acid. When heated above 175^{0}C, it decomposes through the loss of carbon dioxide and water. At the present day most citric acid is produced by using fungi *Aspergillus niger*. Chemical synthesis of citric acid is possible but it is not cheaper as fungal production. Citric acid is recognized as safe for use in food by all major national and international food regulatory agencies (Ali *et al*., 2002). The reasons for choosing *A. niger* over other potential citric acid producing microorganisms are: cheap raw materials (molasses) used as substrate and high consistent yield (Chile, 2002). The significance of *A. niger* is the industrial role that plays in the production of proteins, enzymes and fermentation. It has capabilities of producing heterogeneous proteins, such as human cytokine, interleukin-6. This very useful microorganism is even referred to as an "industrial workhorse'' because of the frequent use in many applications (Hang and Woodams, 1985; El-Holiand Al-Delaimy, 2003; Abdullah-al-Mahim *et al*., 2012). The major advantages of using solid state fermentation rather than submerged fermentation include, the yields are much higher than those in liquid media(Mazaheri, and Nikkhah, 2002; Hang, and Woodams, 1984) and the operating cost are much lower than those for liquid state fermentation (Hesseltine,1972; Rouskas, and Kotzekidou, 1997).

Current disposal of fruit pulp waste poses considerable economic and environmental problems. Therefore, objective of this study was to adopt the use of different fruit pulp waste as a cheap medium for the production of citric acid by using *Aspergillus niger*.

Materials and Methods

Aspergillus niger culture maintenance

The strain of *A. niger* ATCC 16404 was used. The cultures of *A. niger* were maintained on sterilized potato dextrose agar medium pH 4.5 and stored at 5^{0}C in refrigerator.

Preparation of Fruit Pulp Waste (Substrate)

A total six different fruits pulp waste such as Pineapple, Guava, Papaya Beetroot, Wood apple Indian jujube were collected from fruit juice vendors. Out of which Pineapple, Guava, Papaya, Beetroot and Indian jujube fruit pulp waste were prepared by smashing the fruits separately. However, Wood apple pulp was obtained by breaking the hard cover of the fruit. Then, it was smashed to make pulp. Each type of fruit pulp was dried in the hot air current at 60^{0}C for 8hours to reduce the moisture content to 6% and store at room temp (25-30^{0}C). Prior to be used, different fruit pulp were pulverized separately and sieved through 20 mesh sieve (Sukesh *et al*., 2013).

Solid state fermentation

Preparation of inoculums

The inoculums was prepared by adding 10ml of sterile distilled water containing 2 drops of 0.1% Tween 80 to the potato dextrose agar slants of *Aspergillus niger* and the spores of the fungi were carefully scrapped using sterile inoculating needle under aseptic conditions (Pandey, 1992).

Inoculation of medium

A 10ml of inoculum i.e., spore suspension made up of *Aspergillus niger* was added all over the surface of the sterile medium A and medium B into the two conical flasks by using sterile pipette under aseptic conditions. The flasks were kept in a try containing water and incubated at room temp (min.22^{0}C- max 32^{0}C) for 8 days. After incubation sufficient amount of sterile water was added to the flask to get the slurry of the inoculum and it was stirred for 1 hour on rotary shaker to extract Citric acid. The slurry was filtered through Whatmann filter paper no.42. Now the medium was washed with sterile distilled water so as to extract all citric acid. Final volume of the extract was made up to 250 ml with distilled water for the estimation of Citric acid from the extract (Narayanamurthy *et al*., 2008).

Estimation of Sugar by Phenol Sulphuric Acid Method

Total residual sugar of waste fruit pulp powder of six different fruits was estimated calorimetrically by phenol sulphuric acid method. A double beam UV/Vis scanning spectrophotometer was used for measuring colour intensity at 490 nm (Dubois *et al*., 1956).

Estimation of Citric Acid by Pyridine-Acetic Anhydride Method

Anhydrous citric acid' was estimated using pyridine-acetic anhydride method as reported by Pucher *et al*., 1936; Saffran and Denstedt, 1948; Marrier and Boulet, 1958.

Result and Discussion

Sugar concentration in the dry pulp is estimated by phenol sulphuric acid method by comparing standard graph of known sugar concentration for the proper judgement of production of Citric acid.Sugar concentrations in different substrates i.e., dry pulp powder of Wood apple, Beetroot, Indian jujube, Pineapple, Papaya and Guava were estimated and results were noted. The initial sugar concentration varied from 3.2 to 4.0 g/kg of dry pulp powder of the fruits (Table 1) (Fig. 1).

Production of citric acid was carried out in the presence and absence of methanol. As methanol found to be contain stimulatory effect for citric acid production. It was found that the maximum citric acid production occurred in the medium containing dry pulp powder of Pineapple i.e., 3.25 g/kg of citric acid per kg of the substrate used in the medium without methanol while the production of citric acid increased up to 5.25g per kg of the substrate used in the medium with methanol. Citric acid production was found to be 0.75, 2.63, 2.75, 1.5 and 1.75g per kg of the substrate in the medium without methanol with dry fruit pulp powder of Wood apple, Beetroot, Indian jujube, Papaya and Guava respectively (Table 2) (Fig. 2). Thus, it was found that methanol markedly enhanced production of citric acid from fruit pulp waste powder. The addition of methanol at a concentration of 4% (V/V) resulted in the production of 5.25g per kg of Citric acid from Pineapple pulp powder by using *Aspergillus niger*.

Fruit waste is one of the most abundant and locally available agricultural wastes. Fruit waste has high sugar content that can be utilized by microorganisms. In the present study sugar concentration in the substrates i.e., waste pulp powder of Wood apple, Beetroot, Indian jujube, Pineapple, Papaya and Guava were found to be varied from 3.2 to 4.0 g/kg of the substrate (Table 1).The dry pulp investigated for the amount of sugar present, used for the production of citric acid. The production is coupled with the analysis of yield with presence and absence of methanol. It was found that the highest yield obtained with the Wood apple pulp in presence of methanol 5.5 g of citric

Table.1 The optical density and initial sugar concentration of different fruit pulp waste

Sr. No.	Substrate	O.D. of Total Sugar	1:2 Dilution of O.D. of Total Sugar	Concentration of X2 (μg)	Initial Sugar Concentration (g/kg)
1	Woodapple	1.57	0.78	32	3.2
2	Beetroot	1.90	0.95	40	4.0
3	Indian jujube	1.87	0.93	38.5	3.85
4	Pineapple	1.72	0.86	36	3.6
5	Papaya	1.65	0.82	34	3.4
6	Guava	1.95	0.97	40	4.0

O.D. = Optical Density, X2= Dilution Factor

Table.2 The optical density and citric acid yield from different fruit pulp waste with and without methanol addition

Sr. No.	Substrate	O.D. of Citric Acid (420 nm)		Concentration of Citric Acid (mg)		Concentration of Citric Acid (g/kg)	
		WOM	WM	WOM	WM	WOM	WM
1	Woodapple	0.34	0.68	0.11	0.22	0.75	5.5
2	Beetroot	0.33	0.42	0.10	0.13	2.63	3.37
3	Indian jujube	0.34	0.49	0.11	0.16	2.75	4.0
4	Pineapple	0.40	0.65	0.13	0.21	3.25	5.25
5	Papaya	0.18	0.47	0.06	0.15	1.5	3.75
6	Guava	0.23	0.59	0.07	0.19	1.75	4.75

O.D. Optical Density, WOM= Without Methanol, WM= With Methanol

Figure.1 Initial sugar concentration from different fruit pulp waste powder by using *Aspergillus niger*

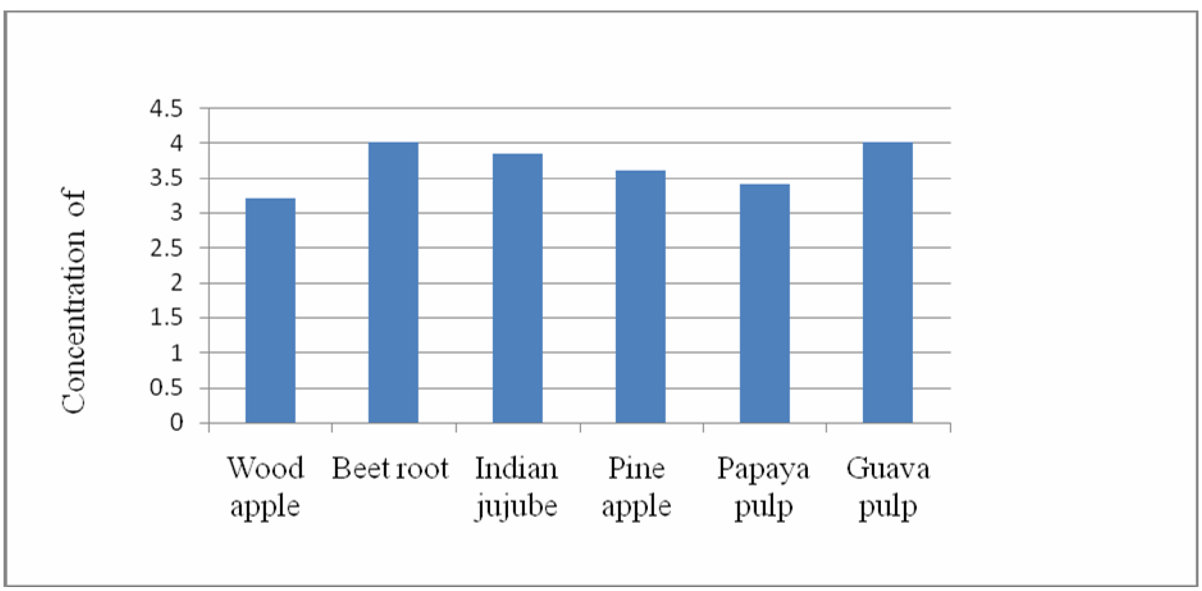
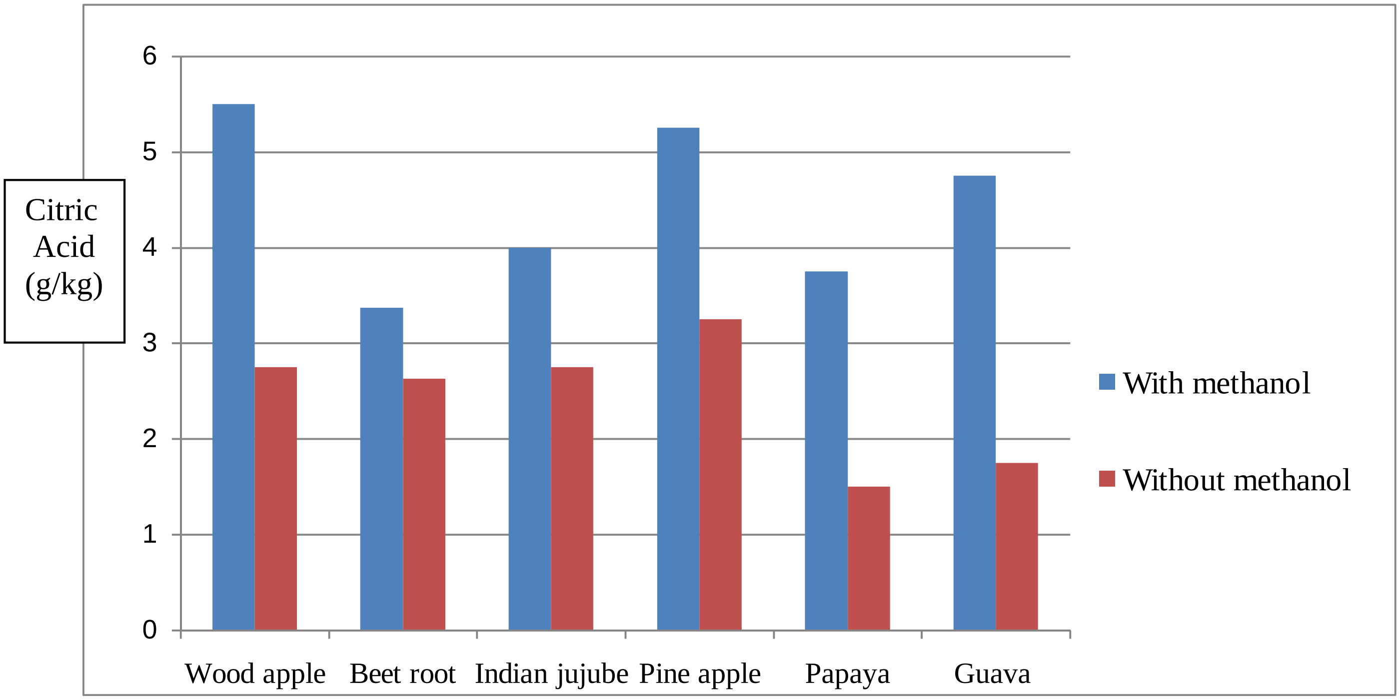


Figure.2 Citric acid yield from different fruit pulp waste powder with and without methanol



acid per kg. Following to it Pine apple and Indian jujube produced 5.25 and 4.0 g of citric acid per kg respectively where as highest yield obtained with the Pineapple pulp in absence of methanol 3.25 g of citric acid per kg. Following to it Woodapple and Indian jujube produced 2.75 g of citric acid per kg in absence of methanol (Figure.1). This study was correlated with that of Femi-ola *et al*., (2013) and Sukesh *et al*.,(2013) in respect of the addition of 1% methanol for increased yield of citric acid using different fruit pulp waste in presence of fungi.

On the same side when a solid state fermentation was developed for citric acid production from pineapple waste by Kareem *et al*., (2010) using *Aspergillus niger* KS-7,the medium which was supplemented with methanol (2% v/v) has given highest citric acid production (60.61 g/kg) with different concentration of glucose, sucrose, ammonium nitrate and ammonium phosphate used. From the above observation it is clear that the availability of methanol is the major controlling factor in the final yield of citric acid supplementation with methanol because it regulates the citric acid production positively. Pineapple pulp was found to be the best suitable substrate for the production of citric acid by *A. niger* ATCC 16404 whereas citric acid production was minimum with the pulp of Papaya and Guava. Methanol was found to have enhancing effect on citric acid production with all the fruit pulp powder tested. Interestingly methanol has more enhancing effect on citric acid production with pulp of Papaya and Guava however citric acid production was minimum in absence of methanol as compared to other fruit pulp tested.

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