



RESEARCH ARTICLE

UTILIZATION OF MANGO PEELS FOR CITRIC ACID PRODUCTION BY USING *ASPERGILLUS NIGER*

Satheesh kumar, S., *Sivagurunathan, P., Uma, C. and Muthulakshmi, K.

Department of Microbiology, faculty of science Annamalai University, Annamalai Nagar, Tamil Nadu, India

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ABSTRACT

Utilization of agro wastes and by products for the production of organic acid increasing worldwide. The demand for citric acid is increasing due to their wide application in the various industries like food, beverage, pharmaceutical and cosmetic. The present study was aimed to utilize mango peel as a substrate for the production of citric acid using *Aspergillus niger* isolates. Among the 6 isolates obtained from the fruit waste dumped soil, the isolate *Aspergillus niger*. I recorded highest yield of citric acid (4.52%).

Key words: mango peels, *Aspergillus niger*, Citric acid.

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INTRODUCTION

Mango (*Mangifera indica* L.) belongs to the family *Anacardiaceae*, is the king among tropical fruits and is greatly relished for its succulence, exotic flavour and delicious taste in most countries of the world (Bhatnagar and Subramanyam, 1973). It is a nutritionally fruit being a good source of vitamin A, vitamin B, C and minerals. Mango has its origin in India and approximately a thousand different types of mango fruits are produced in the country. Annual production of mango in India is 15.19 million tons (FAO, 2011). About 20% of total world mango production is utilized to produce value added products like mango pulp, juice, squash, nectar, fruit leathers, jam, canned slices, peels, pickles, chutney, dried powder etc. Which have most popular in the world (Rameshwar et al., 1979., Kalra et al., 1981., Ajila et al., 2007). During processing of mango, byproducts such as peel and kernel are generated. Peel contributes about 7-24% of the fruit (Wu et al., 1993). As peel is discarded as a waste and cause pollution due to their high carbohydrate content (20.80 – 28.20%) in dry weight samples of mango peel. (Ajila et al., 2007; Somda et al., 2011). Citric acid (2 – hydroxyl – propane – 1,2,3 – tricarboxylic acid) is a organic acid, soluble in water with pleasant taste, and is the most important in food and other industries. The production of citric acid has increased rapidly due to their demand in the world. Citric acid is widely utilized in the food, beverages, chemical, pharmaceutical and cosmetic industries. Citric acid derives its name from the Latin citrus, the citron tree, the fruit of which resemble a lemon.

Citric acid can be produced via ①. Mechanical ②. Chemical ③. Fermentation methods, production through mechanical and chemical method are highly expensive. Production of citric acid using refined sugars by fermentation process are expensive and can be replaced by various cheap, easily available agro-industrial wastes or by-products (Adhm, 2013). Many microorganisms have been employed for citric acid production including bacteria and fungi such as *Bacillus licheniformis*, *Arthobacter Paraffinens* and *Corynebacterium* spp., *Aspergillus niger*, *A. carbonarius*, *A. aculeatus*, *A. awamori*, *A. fonsceaeus*, *A. foetidus*, *A. phoenicis* and *Penicillium janthinellum*; and yeasts such as *Candida tropicalis*, *C. oleophila*, *C. guilliermondii*, *C. citroformans*, *Hansenula anamola* and *Yarrowia lipolytica*. Among the microbes, *Aspergillus niger* is highly preferred for commercial production because of their ability to utilize various types of substrates and to accumulate large quantities of citric acid (Papagianni 2007). The objective of the present study was to utilize mango peels for the production of citric acid.

MATERIALS AND METHODS

Isolation of citric acid producing fungi from 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 and spread plate method was performed using the dilution 10^{-3} , 10^{-4} and 10^{-5} on PDA medium and incubated at 25°C for 5 days. After incubation the block color colonies were suspected as *Aspergillus niger* and selected for citric acid production.

*Corresponding author: Sivagurunathan, P.

Department of Microbiology, faculty of science Annamalai University, Annamalai Nagar, Tamil Nadu, India.

Identification of fungal isolates

The citric acid producing fungal isolates were streaked on to CDA plates ; incubated at room temperature for 5 days .After incubation , the isolates were identified based on their cultural characteristics and microscopic observation using Lacto phenol cotton Blue(LPCB) Staining method.

Collection of substrates

The mango peels samples were collected from local fruit market and juice shops in and around Chidambaram.

Pretreatment of substrate

The collected fruit peel were dried in a hot air oven at 60°C for 2 hrs. Then the dried samples were cut into small pieces and they were used for citric acid production.

Inoculum Preparation

The strain of *Aspergillus niger* were grow on CDA plates at a temperature of 25°C for 5 to 7 days. After incubation, the spore suspension was prepared with tween 80 solution. The suspension containing 10^8 spores/ml was used as a inoculum for citric acid production (Kdajjah-Al-kadir and Mohd, 2011).

Fermentation

The basal medium for the fermentation was prepared by adding 5g of pretreated substrate in (5%) Fermentation experiments were conducted in 250ml Erlen mayer flasks, 100 ml medium with 5% substrate was taken in separate flasks and sterilized at 121°C ,15lbs pressure for 15 minutes. A spore suspension containing 10^8 Spores/ml of *Aspergillus niger* were inoculated and incubated at 30 °C for 5 days . After fermentation the media were diluted with addition of 100ml of distilled water and the filtered by using Whatman No.1 filter paper. The resulting filtrate was used for the determination of citric acid by titration method using 0.1N NaOH and phenolphthalein as indicator (AOAC 1995).

RESULT

The fruit waste such as mango peel were collected, dried and cut into 2mm sized particles. These particles were used throughout the study. Serial dilution technique was followed to determine the fungal load of fruit waste dumped soil. Black colours colonies were suspected as *Aspergillus* sp and were selected for screening of citric acid production. About6 isolates were obtained from the fruit waste dumped soil and they were identified based on cultural and morphological characteristics using LPCB test. Among the six isolates obtained from fruit waste dumped soil, *Aspergillus niger* 1 was the better producer of citric acid (4.2 %). This was followed by *Aspergillus niger* VI (2.3%), *Aspergillus niger* II (2.0%), *Aspergillus niger* III (1.5%), *Aspergillus niger* V (1.8%), *Aspergillus niger* IV (0.5%). The fungal isolates were identified through morphological characteristics by LPCB staining method and colony characteristics on CDA plates. Narayanamurthy *et al.*, (2008) isolated *Aspergillus niger* RCNM17 from the soil of fruit dump outside a wholesale fruit stalls.

Table 1. Citric acid production by *Aspergillus niger* isolates

S.No	Isolate	Citric acid production %
1.	<i>Aspergillus niger</i> I	4.52
2.	<i>Aspergillus niger</i> II	2
3.	<i>Aspergillus niger</i> III	1.5
4.	<i>Aspergillus niger</i> IV	0.5
5.	<i>Aspergillus niger</i> V	1.8
6.	<i>Aspergillus niger</i> VI	2.3

DISCUSSION

Roher *et al.*, (1983) reported that citric acid can be produced by fermentation process using species of microorganisms named *Aspergillus niger*, a fungus which was used commercially for the first time in 1923. Many of them have been studied with SSF techniques for their potential use as substrates for citric acid production in citric acid production (Vandenberghe *et al.*, 2000). Most of the citric acid produced by fungal strains of *Aspergillus niger* fermentation. Chemical synthesis is possible but it is not cheaper than fungal fermentation (Yigitoglu 1992). Various types of yeast and bacteria are capable of producing citric acid, but *Aspergillus niger* could utilize cheapest raw materials and give high yield of citric acid (Kapoor *et al.*, 1982). The main advantages of using *Aspergillus niger* are its ease of handling, its ability to ferment a variety of cheap raw materials and high yield (Imandi *et al.*, 2007). The present study was mainly focused about the fruit waste mango peel as potential substrate for the production of citric acid using *Aspergillus niger* isolats. Among the six isolates obtained from fruit waste dumped soil, *Aspergillus niger* 1 was the better producer of citric acid (4.2 %). This was followed by *Aspergillus niger* VI (2.3%), *Aspergillus niger* II (2.0%), *Aspergillus niger* III (1.5%), *Aspergillus niger* V (1.8%), *Aspergillus niger* IV (0.5%). The fungal isolates were identified through morphological characteristics by LPCB staining method and colony characteristics on CDA plates. Narayanamurthy *et al.*, (2008) isolated *Aspergillus niger* RCNM17 from the soil of fruit dump outside a wholesale fruit stalls.

Sukesh *et al.*, (2013) isolated three fungal isolates, among them only one isolate of *Aspergillus niger* produced maximum citric acid from different agronomic waste *viz.*, grape, orange, apple, vegetable, tapioca and coconut husk. In our study, the isolate *Aspergillus niger* 1 produced maximum amount of citric acid (4.25%), from mango peel. They production of citric acid by utilizing different fruit waste such as grape, pomace, orange peel, kiwi fruit peel (Hang and Woodams., 1986,1987), pineapple waste, citrus waste and banana peel were documented by many authors (Tran and Mitchell., 1995;Shojaosadati and Babaoipour., 2002). Dhandayuthapani *et al.*, (2008) used different varieties of banana peel for producing citric acid by *Aspergillus niger*.

Conclusion

Based on the result of the present study, it could be concluded that the fruit waste i.e. mango peel can be used as a potential substrate for production of citric acid. The study explored the efficient way to minimize environmental pollution due to dumping of fruit wastes. Further studies like optimization of different fermentation parameters could improve the yield of citric acid.

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