



CITRIC ACID PRODUCTION ON DIFFERENT FRUIT PEELS

M. Kaleeswari¹* G. Lakshmi Priya² and S. Maheswari³

¹Assistant Professor, Department of Microbiology, The Standard Fireworks Rajaratnam College for Women (Autonomous), Sivakasi – 626 123.

^{2,3}BSc Microbiology, Department of Microbiology, The Standard Fireworks Rajaratnam College for Women (Autonomous), Sivakasi – 626123.

***Corresponding Author: M. Kaleeswari**

Assistant Professor, Department of Microbiology, The Standard Fireworks Rajaratnam College for Women (Autonomous), Sivakasi - 626 123.

Article Received on 14/12/2017

Article Revised on 02/01/2018

Article Accepted on 23/01/2018

ABSTRACT

Citric acid is typical compound produced by natural fermentation of fruit peels of sound quality. In the present study the potential citric acid producing fungus *Aspergillus niger* was obtained and isolated from lemon sample. Four different raw fruit peels were used as a substrate for the citric acid production by using *Aspergillus niger*. The sucrose is used as a carbon source. The fruits such as pine apple, grapes, sweet lime, and gooseberry were selected for the citric acid production. Among these fruit peels substrates the grapes gave the better yield of citric acid in fermentation process (66%) when compared with other fruit peels. Next to that goose berry have good citric acid content (49.8%). Then the citric acid in the fermented sample is crystallized by using precipitation method. It is done by mixing calcium hydroxide and sulphuric acid. After that it was centrifuged. The pellet was collected. It was dried and obtained a crystalline form of citric acid. The citric acid is used for application studies such as, adhesive agent on the wood that is the decorative particle that may attach to wood when paste with citric acid and stain removal on clothing.

KEYWORDS: Fermentation, citric acid and *Aspergillus niger*.

INTRODUCTION

Citric acid (2-hydroxy propane, 2, 3-tricarboxylic acid C₆H₈O₇) is universal in nature. It is a natural preservative which is present in citrus fruits. It is also used to include an acidic or sour taste to food and drinks. Citric acid is 6 carbons containing Tricarboxylic Acid which was first isolated from lemon juices. Citric acid is an intermediate of Tricarboxylic acid cycle which is obtained when carbohydrates are oxidised to carbon IV oxide. (Prabha and Rangaiah 2014).

A considerable interest has been shown in using agricultural products as alternative sources of carbon and their wastes such as maize, apple and grape pomace, pineapple, mandarin orange and brewery wastes, citrus, kiwi fruit peel, pumpkin, jackfruit and spoiled coconut for citric acid production by *Aspergillus niger*. Citric acid is a versatile and harmless alimentary additive. It is accepted worldwide as GRAS (generally recognized as safe), approved by the Joint FAO/WHOM, Expert Committee on Food Additives. The food and pharmaceutical industries utilize citric acid extensively because of its general recognition of safety, pleasant acid taste, high water solubility and chelating and buffering properties.

In the pharmaceutical industry, citric acid is used as a preservative for stored blood, tablets, ointments, and cosmetic preparations. In the chemical industry, it is used as an antifoam agent and for the treatment of textiles. Recently, major production of citric acid was conducted via microbial fermentation, as it was economical and easy to handle. According to our previous researches, citric acid had good bond performance for wood-based molding, and its main bonding mechanism was ester linkages between the carboxyl groups of citric acid and hydroxyl groups of wood components. (Umemura *et al.*, 2012)

MATERIALS AND METHODS

Isolation of Fungi

The spoiled lemon sample was used for the isolation of *Aspergillus niger* for the citric acid production. The loopful of inoculums was taken and inoculated in potato dextrose agar medium. Then the plates were incubated for 3 days. After incubation the black coloured powdery colonies were isolated and used for the citric acid production.

Identification of Fungi

The isolated fungi were identified by using Lactophenol Cotton Blue staining. The spores and hyphae were observed under microscope.

PROCESSING OF CITRIC ACID

Citric Acid Production

The fruit peels (pine apple, sweet lime, grapes, and gooseberry) were dried and powdered was used as substrate for fermentation. The 20g of powdered peels were diluted in 100 ml of distilled water in 2 different conical flasks. It was followed by addition of carbon source (sucrose) and nitrogen source (Ammonium nitrate) in one flask of each sample and the inoculums of *Aspergillus niger* is inoculated. Another medium simply contain inoculums of *Aspergillus niger* in each sample. It was mixed well. Then they are incubated in shaker incubator for 5 day for fermenting the medium.

Purification

After incubation, the citric acid was purified by Filtration method by using Whatmann no.1 filter paper. This is simple and highly effective method for purification process.

Crystallization

The fermentation broth was taken in a centrifuge tube. It was followed by the addition of calcium hydroxide (lime). The citric acid is transformed into tri-calcium citrate tetra hydrate, which is lightly soluble. The precipitation was formed in this solution. Then the precipitate is recovered by filtration. After that it was treated with sulphuric acid which forming calcium sulphate (gypsum). It was centrifuged. The pellet was taken. Anhydrous citric acid is obtained at crystallization temperature higher than 36.5 °C.

Estimation of Total Acidity

To estimate the total acidity, 10 ml of fermented sample was taken. Then 10 ml of distilled water was added to it. It was followed by the addition of phenolphthalein solution (1%). It was mixed well. Titration was done by the first persistent pink colour with 0.1N Sodium hydroxide.

RESULT

Isolation of Fungi

The black colour filamentous colony was seen. That colour was primarily confirmed as the colonies was comes from the *Aspergillus* species.

ESTIMATION PROCESS

TABLE 1: Estimation of citric acid.

S.No.	Sample	Citric acid production (with sucrose) %	Citric acid production (control) %
1.	Pine apple	11	1.5
2.	Sweet lime	22	12.6
3.	Grapes	66	14.3
4.	Gooseberry	49.8	13.2

Identification of Fungi

The brown coloured spores and mycelium were observed under microscope.

Citric Acid Production

The citric acid was produced by using different fruit peels. The fermentation process was carried out in shaking incubator for 5 days for the citric acid production. After 5 days of incubation, the fermented medium has a citric acid. It carried out as sample with sucrose and control.

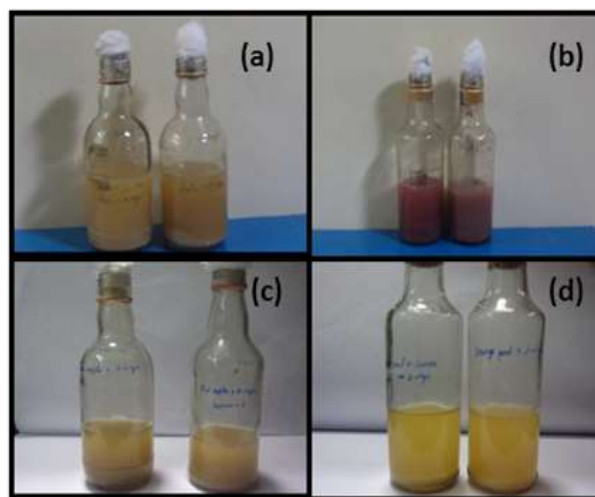


Fig 4. Citric acid on (a) grapes peels (b) pine apple (c) gooseberry (d) sweet lime

PURIFICATION

The citric acid was purified by filtration process. Whatmann no.1 filter paper was used. By this process, the fermented samples were filtered and the turbid was removed.

CRYSTALLIZATION

The citric acid in the fermented sample was crystallized by using precipitation process. It was precipitated with calcium hydroxide and sulfuric acid and centrifuged. The pellet was collected and dried. This result in citric acid is obtained as white, crystallized powder form.

APPLICATION OF CITRIC ACID

In our present study, the citric acid was used for various applications such as removing stain in cloth, removing limescale on tap, and used as adhesive agent on wood materials.

DISCUSSION

A. niger has remained the organism of choice for commercial production because it produces more citric acid per time unit (Soccol *et al.*, 2006). The citric acid was produced by using various fruit peels such as pine apple, goose berry, grapes and sweet lime peels. The citric acid was obtained by fermentation process by using the strain *Aspergillus niger*. In the fermentation process, sucrose is used as the carbon source. The yield of citric acid in fermentation by *A. niger* is strongly dependent on the type and concentration of carbon source. (V.Iralapati, V., S. Kummari 2014). The citric acid was crystallized by using precipitation method (Kareem *et al.* 2010).

CONCLUSION

The present study concluded that, to isolate and screen the citric acid producing *Aspergillus niger* strain. A maximum citric acid production in grapes peels as 66 mg %.

ACKNOWLEDGEMENT

We thank Management of the Standard Fireworks Rajaratnam College for women, Sivakasi for the facilities provided to make this work possible.

BIBLIOGRAPHY

1. Kareem, S.O., I. Akpan and O.O. Alebiowu. Production of citric acid by *Aspergillus niger* using pine apple waste. Malaysian Journal of Microbiology, 2009; 6(2): 161-165.
2. Prabha, S.M. and G.S. Rangaiah, Effect of different alcohol at various concentrations on citric acid fermentation using *Manikars zapota* and its peels as a carbohydrate source. Int. J. Pure App. Biosci., 2014; 2(6): 112-119.
3. Raj and Chaudhary. Citric from biowaste: challenges and prospects BioEvolution ISBN 978-81-925781-3-2, 2014; 48-50.
4. Soccol, C.R., P.S.I. Vandenberghe, C. Rodriguez, and A. Pandey. New perspective for citric acid production and application. Food technology biotechnology, 2006; 44(2): 141-149.
5. Umemura K, T. Ueda, S.M. Sasa, S. Kawai Application of citric acid as natural adhesive for wood. J Appl Polym Sci., 2012; 123: 1991–1996.
6. Vinusha Iralapati, V., S. Kummari. Production of Citric Acid from Different Fruit Peels Using *Aspergillus niger*. International Journal of Scientific Engineering and Research, 2014; 3: 129-130.