STUDY OF PHYSICOCHEMICAL EFFECT OF CITRIC ACID MADE FROM ORANGE PEELS AND *ASPERGILLUS NIGER*

[1]Damini R.Motwani, [2]Rahul L.Meshram
Assistant Professor , Deptt. Of Biochemistry, DRB Sindhu Mahavidyalaya, KTR Marg, Panchpaoli Nagpur 440017 Maharashtra (India)
Email:daminimotwani7@gmail.com[1]

ABSTRACT

This work was aimed to study the physicochemical and preservative effects of citric acid produced from orange peels using *Aspergillus niger*. Fresh orange peels were washed, sundried and milled into flour, after which it was inoculated with *Aspergillus niger* in a nutrient medium and fermented for 6 days at 30 °C. Citric acid was extracted from the fermented substrate (1200 mL) and total yield was 418 g of citric acid. Physico-chemical properties of the extracted and commercial citric acid were compared and the results were; pH (2.80 and 2.66), TTA (0.77% and 0.75%) and moisture content (2.40% and 1.8%) respectively. Appearance of both samples were crystalline with the extracted citric acid having a more off white colour. The preservative effects of commercial citric acid and extracted citric acid from orange peels were compared. This implies that citric acid extracted from orange peels had better preservative effects compared to the commercially produced citric acid.

Keywords: Citric acid, Aspergillus Niger, orange peel and Physicochemical effect.

1. INTRODUCTION

Citric acid (CA) is a common organic acid utilized in various industries. There is a worldwide demand for citric acid consumption due to their many industrial applications [1]. All microbial cells utilize citric acid as an intermediary in the Tricarboxylic acid cycle (TCA), which is essential to their metabolism [2]. This all-natural component promotes good digestion and kidney function, helps with detoxification, and keeps energy levels stable [3]. It is used to balance the sweetness in soft drinks, juices, and other beverages because of its somewhat tangy and refreshing flavor [4].

Food waste, including leftovers and precooked food, is a biodegradable waste that is released from a variety of sources, such as homes, food processing businesses, and the hospitality industry. Nearly 1.3 billion tonnes of food, including fresh fruits, vegetables, meat, bread, and dairy products, are wasted within the food supply chain, according to the FAO [5].

According to Lakshmi [6], waste management is an integral part of the supply chains that our global economy relies on. Due to exponential growth of population there has been a remarkable increase in everyday waste wherein improper treatment and disposal cause serious socio-economic downturns. This became a major concern to many of developing countries where safe and sustainable practices are scarce and waste management has not been adequately regulated. However, certain wastes may eventually become resources valuable to others once they are removed from the waste stream [7].

Citric acid exists in a variety of fruits and vegetables. Most notably, citrus fruits, lemons and limes have particularly high concentrations of the acid. According to Penniston et al. (2008)[8], it can make up as much as 8% of the fruits' dry weight and roughly 47 g/L in their juices.However, under certain conditions of drastic nutrient imbalance, fungi, yeast, and bacteria produce citric acid in excessive amounts [9][10].

Significant attention has been directed towards the proficient use of waste and its management.

Several methods of adding value to agricultural waste have been assessed for this aim, taking into account the principles of recycling, pollution management, and reducing environmental littering [11].With the increase in consumption demand, there is need for substrate alternatives in the manufacture of citric acid that is cheaper and available than the present substrate, especially those of our waste products.

Orange peels have several nutritional benefits; they can be used in a variety of food applications; and they can help improve food security in developing countries. However, their utilization as an industrial raw material is still quite low [12].

The first patent for Citric acid production (CAP) by *Aspergillus niger* utilizing sugar solutions was reported in 1913 [13]. Its high potential for converting various raw materials to valuable products, such as lipase, oxalic acid and microbial lipids, has been demonstrated in previous studies [14][15]. In addition, citric acid and many other products of *A. niger* have been in use for many decades and are considered GRAS (generally regarded as safe) by the U.S. Food and Drug Administration [16].

Orange peels are comprised of mainly carbohydrates with some fractions of protein, fat, and nutrients such as calcium, potassium, magnesium, zinc, and vitamins [17]. The composition of orange peels represents an opportunity to utilize it as a suitable substrate for deriving citric acid via solid state fermentation, which becomes a local means of producing citric acid.

It is clear from market trends that there would be a spike in the demand for citric acid globally, as reported by Pau et al. (2015)[18]. There is a rapid increase in the number of food and beverage industries with little to no known companies generating the acid there. Therefore, it's imperative to maximize the production of citric acid by seeking for substitutes that are more affordable, eco-friendly, and produce more than the existing techniques.

2. MATERIAL AND METHODS

2.1 Materials Procurement

Orange peels obtained from santra market of Nagpur.

2.2 Preparation of Orange peel

Orange peels were sundried for two weeks followed by size reduction which was done using a grinding machine to turn into powdery form. It was then sieved, packaged in a sterile air tight container, labeled and stored under dry conditions at 30 °C for laboratory analyses

2.3 Inoculum Preparation

The isolate was incubated in Sabouraud dextrose agar (SDA) at 30 °C for 6 d
inoculums used for citric acid production was prepared by taking 2 fungal plugs with a diameter 8 mm and inoculating a 100 mL broth medium, a modification of Perwitasari*et al.*(2021)[19].

2.4 Production of citric acid

Solid state fermentation was carried out using modified methods optimized by Amenaghawon *et al*. (2023)[20]. Firstly, 20 g of the substrate (milled orange peels) was dispensed in a 250 mL Erlenmeyer flask (three flasks were used per experimental run). The substrate was then mixed with a nutrient medium. The composition of the nutrient medium (g/L) was $ZnSO_4•7$
H_2O (0.002
H_2O (0.
MnSO4•H2O (0.006). The flasks were thoroughly shaken to mix the contents and then cotton-plugged before autoclaving at 121 °C for
15 minutes to sterilize the samples. The flasks were then cooled and inoculated with the inoculum (2 mL) and incubated for six days at 30 °C in a rota
fermentation the solid substrate was diluted with 100 mL of distilled water and agitated to enhance dissolving process after which it was filtered off using Whatman filter paper and the filtrate used for the analysis.

The concentration of citric acid produced during fermentation was determined using the pyridine acetic anhydride method as reported by Marrier and Boulet (1958)[21]. This was executed by adding 1 mL of the filtered fermentation broth along with 1.30 mL of pyridine and 5.7 mL of acetic anhydride in a test tube. The test tube was then placed in a water bath at 32 °C for 30 min. The absorbance of the sample was measured at 405 nm using a UV-Vis spectrophotometer.The concentration of citric acid in the sample was determined from a citric acid calibration curve which was prepared from known concentrations of citric acid.

2.5 Citric acid Recovery

First, 500 mL of the fermented samples was weighed into a 1000 mL beaker and 500 mL volume of $CaCl_2$ of 40.7% (w/v) was added to the sample, and heated in boiling water bath for 30 minutes. Double displacement reaction takes place between the fermented sample and $CaCl_2$ solution which resulted in the formation of precipitated at the bottom. The resultant mixture was filtered using vacuum filtration and the residue was washed with 100 mL of hot water (100 °C) to remove the impurities and by products. During the addition of hot water, the residue was stirred well until it attained a neutral pH of 7. The resultant residue was then dried in hot air oven. The dried filtrate (calcium citrate) was acidified with 250 mL volume of dilute H_2SO_4 of 1.9 M, and heated at 60^0C with simultaneous mixing with glass rod. When calcium citrate and sulfuric acid were mixed, calcium sulfate precipitated at the bottom leaving behind citric acid solution at the top and the mixture was vacuum filtered. Finally, the citric acid was crystallized from its aqueous solution by evaporative crystallization, the yield of citric acid crystals estimated gravimetrically.

2.5 Physicochemical Properties

The moisture, moisture content, pH, yield and total titratable acidity (TTA) of the processed and commercial citric acid was determined using AOAC (2010)[22].

3. RESULTS AND DISCUSSION

3.1 Physicochemical properties of Citric acid

The physicochemical properties of produced and commercial citric acids are presented in Table 1. From observation the pH values of both samples are acidic, with the produced citric acid having a slightly higher pH of 2.80, and the commercial citric acid having a slightly lower pH of 2.66.

The percentage of acidity (TTA) is slightly higher in the produced citric acid (0.77%) compared to the commercial citric acid (0.75%). The physical properties of the citric acid derived from the substrate as well as those from commercial source are similar as they were both crystalline and granular in nature, while a slight difference in colour was noticed with the commercial citric being more white and the produced citric acid a more off-white color. The moisture content of the produced citric acid was observed to be slightly higher than that of the commercial citric acid.With produced citric acid having a moisture content of 2.40% while that of the commercial citric acid was observed to be 1.8%. Although the moisture content and color of the fermented citric acid were observed to have improved over the course of time.

Table 1:Physicochemical properties of citric acid

Properties	Produced Citric acid	Commercial Citric acid
Colour	Off White	White
Appearance	Crystal	Crystal
pH	2.80	2.66
TTA in percent	0.77	0.75
Moisture in percent	2.40	2.80

4. CONCLUSION

This study revealed that citric acid produced from the fermentation of orange peels can compete favorably with the commercially synthesized citric acid and its utilization will facilitate both the large scale production of this commercially valuable organic acid and also aid in cleaning up of our messed environment, thereby reducing environmental pollution and improving on waste recycling.

The utilization of orange peels for citric acid production could encourage the development of local industries in regions where yam cultivation is prominent. This can lead to the establishment of small-scale processing units, job creation, and the overall development of the agricultural and biotechnology sectors in the area.

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