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Estimation and economic analysis of citric acid extracted from vegetative wastes collected from Vellore

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ABSTRACT

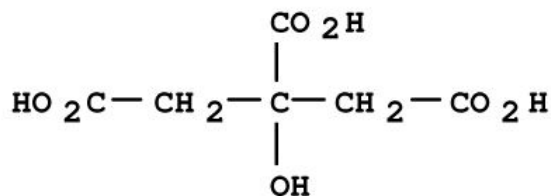
Citric acid is a weak organic acid that occurs naturally in various fruits and vegetables, especially in the citrus fruits. Since olden days till now, citric acid is produced by any one of the three methods – fermentation, synthesis and extraction from citrus fruits. Nearly 50% of the total citric acid produced worldwide is used as a flavouring agent in beverages; its acidic pH allows it to be a good preservative as well as aiding in ripening processes when cheese is converted to mozzarella and also adjust pH in brewing industry; its powder form is used as a sour alternative to many dry foods. Citric acid also acts as a dietary supplement - owing to its acidic pH because it creates a suitable environment for mineral and vitamin adsorption. Citric acid has immense medicinal values like it prevents kidney stones; its antioxidant property helps to regenerate skin tissue, slow down the ageing process and also depigments stained skin; it also reduces nausea and prevents tonsillitis. With so many beneficial aspects and uses of citric acid, its demand has and will increase over the years and so should its supply. In terms of production, the extraction method is uneconomical (only 7% of world production) and demand outweighs the supply of citrus fruits; the synthetic process is expensive, low yielding and hazardous. The fermentation method is by far the best adopted method for citric acid production (gives 90% of world production). The primary aim of this project is to produce citric acid from non citrus vegetable wastes like rotten fruits and vegetables found in municipal garbages and then to compare the yield with a standard citric acid in order to find out the viability of this technique in industrial scale. A comparison of the net yield of citric acid is done in between nine waste vegetables. 156.523 gm/l is found in Brinjal waste followed by potato and other vegetable wastes screening, production strategies and downstream processing of citrate will be envisaged.

Keywords: Citric acid, Krebs Cycle, Downstream Processing, vegetative wastes

INTRODUCTION

Citric acid is a tri-carboxylic acid and is the first metabolic product of Krebs cycle. Chemically it is 2-hydroxy propane-1,2,3- tri carboxylic acid.[14] England witnessed the first commercial production of citric acid in 1896 from Italian lemons until 1919, after which *Aspergillus niger* replaced lemons for industrial production in Belgium. The chemical synthesis of citric acid was carried out by Grimoux and Adams from glycerol, and later from dichloroacetone – but by far, the chemical methods have proved to be non-competitive. However, it was discovered that some organisms, mainly fungi, accumulate citric acid as intermediate/by product during their metabolic actions and with carefully applied methodology, it can be isolated. Wehmer first demonstrated that *Citromyces*, when cultured in a media containing sugar and inorganic salts accumulated citric acid in the media. Since then, many organisms were found to accumulate citric acid as one of their metabolite, for example, *Aspergillus niger*,

Aspergillus awamori , *Aspergillus nidulans* , *Aspergillus saitoi* , *Aspergillus flavus* , *Mucor piriformis* , *Trichoderma viride* etc.



Apart from fungi , various yeasts are also responsible for producing citric acid from n-alkanes and carbohydrates. The following genera of yeasts are mainly responsible for this – *Candida* , *Hansenula* , *Pichia* , *Torula* , *Torulopsis* , *Kloekera* , *Debaromyces* , *Saccharomyces* , *Zygosaccharomyces* and *Yarrowia* .

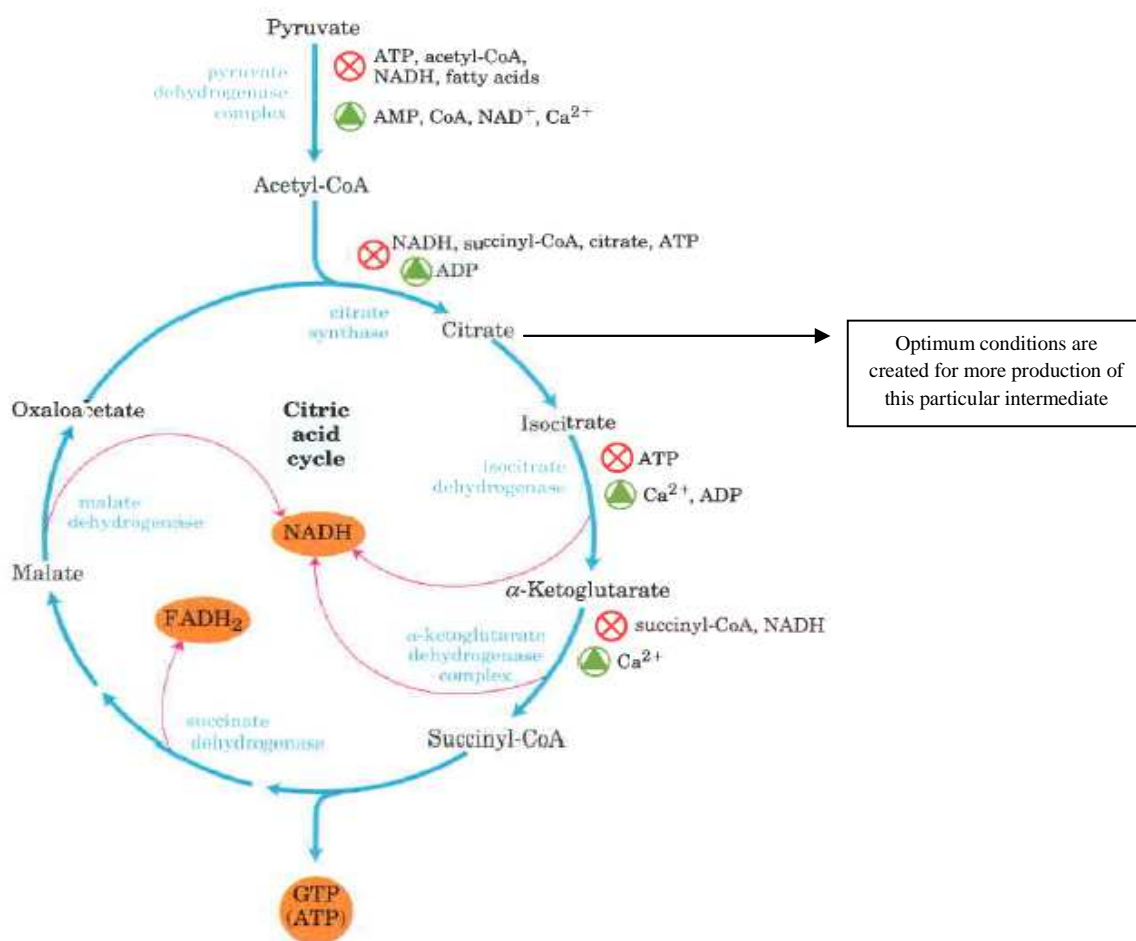


Fig.1 TCA cycle [14]

The chief mechanism operating behind bio-production of citric acid is Krebs cycle or TCA cycle [Fig.1], which is a nearly universal central catabolic pathway where compounds, derived from the breakdown of carbohydrates, fats, and proteins are oxidized and converted to carbon dioxide, and the energy of oxidation is held by the electron carriers FADH₂ and NADH. During aerobic respiration, the electrons are transferred to oxygen and the energy of these electrons are trapped as ATP molecules.

Acetyl-CoA enters the citric acid cycle (in the mitochondria of eukaryotes, the cytosol of bacteria) where it condensates with oxaloacetate to form citrate.

In seven sequential reactions, the citric acid cycle converts citrate to oxaloacetate. For each molecule of acetyl-CoA oxidized by the Krebs cycle, the energy is stored in these molecules -NADH, one FADH₂, and one ATP or GTP.

In commercial scenario of citric acid production, the ability of the fungi or yeast to store citric acid as one of the primary metabolite via TCA cycle is exploited. The culture bed is continuously monitored and accordingly optimum conditions are generated that helps in elevated production of citric acid.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

The following nine vegetable wastes were collected from the Katpadi Market, in Vellore [Fig.2] – Potatoes, Tomatoes, Beet, Cabbage, Lemon, Carrots, Beans, Brinjal and Yam. To prepare the samples for further experimentation [1-6], they were made to undergo aerobic degradation. Each one of the samples was chopped/grinded with knife, mortar and pestle. Samples were kept in separate white colored containers, immersed fully in water, at room temperature such that it undergoes aerobic degradation. Degradation was allowed for 3 weeks, signs of fungal or in general microbial growth was observed eventually in each container.[7,9,10]

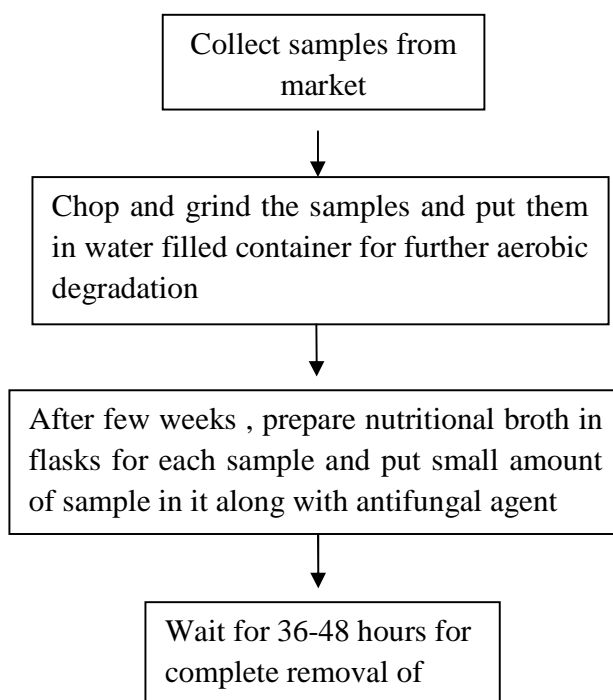
MEDIA EMPLOYED

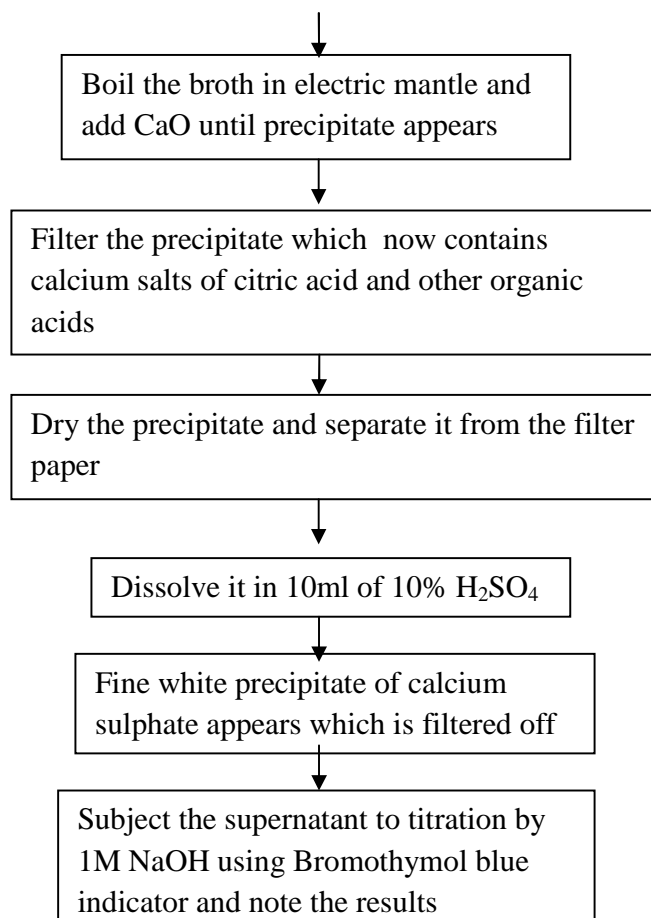
Separate nutritional broth were prepared for every single sample as their respective media and anti-fungal treatment followed afterwards –

About 0.65 gm of “nutrition broth” powder was taken in a conical flask and 50 ml of distilled water was added to it. The mouth of the flask was covered carefully with cotton plug and paper. The flask(s) were put in a polythene packet and were subjected to 121⁰C for 15 minutes sterilization. Next, the flask(s) were brought out from the autoclave, the cotton plug was removed and little amount of samples were added in each of the flask; inside the laminar airflow cabinet under UV-light, near Bunsen flame, for creation of a proper sterile environment.[8] An anti-fungal agent was added in each of the flask(s). (Anti-fungal agent used: fucanazole. Finally, the flasks were left in the incubator for one and a half to two days. [Fig.3]

METHODOLOGY

The whole process is schematically represented by the given chart –





Chemicals:-Fuconazole, 10% sulphuric acid, 1M NaOH, calcium oxide, indicator bromothymol blue, distilled water.
 Apparatus:-Beaker, conical flask, burette, pipette, electric mantle, laminar flow, test tubes, measuring cylinder, Whitmanns filter paper.

PRODUCTION, SEPARATION AND ESTIMATION OF CITRIC ACID

For the downstream processing, 10% sulphuric acid, 1M NaOH (equivalent to 1N NaOH) and CaO were taken. First 10 ml sample from production media was taken and filtered to remove the dead cell mass. The filtrate was boiled and CaO was added till precipitate was obtained [Fig.4]. Then the precipitate was dried at high temperature [Fig.5]. Precipitate was then powdered and 10ml of 10% sulphuric acid was added to it, to regenerate citric acid. The calcium sulphate precipitate thus formed was separated by filtration [Fig.6] and the filtrate was titrated using 1M NaOH solution. Bromothymol blue was used as an indicator [8,9,10]. The solution's colour changes from yellow to blue with the change in pH from acidic to basic respectively [Fig.7]. The concentration of citric acid was estimated by using the Normality Law, i.e.

$$V \times N = V' \times N'$$

Where V=volume of titrand, N=concentration of titrand, V'=volume of unknown analyte, N'=concentration of unknown analyte. To estimate the concentration in terms of grams/lit the values were converted to their corresponding molar values using the formula [11-14]

Normality = n x Molarity (where n=3 for citric acid)

To obtain the molar values in gm/lit the given formula is used. Concentration in gm/lit = Molarity x molecular weight of citric acid [Table 1]

[Molecular weight of citric acid is 192.124 gm/mole] . All the concentration values were plotted in a standard bar graph for schematic representation of all the values [Table 2]

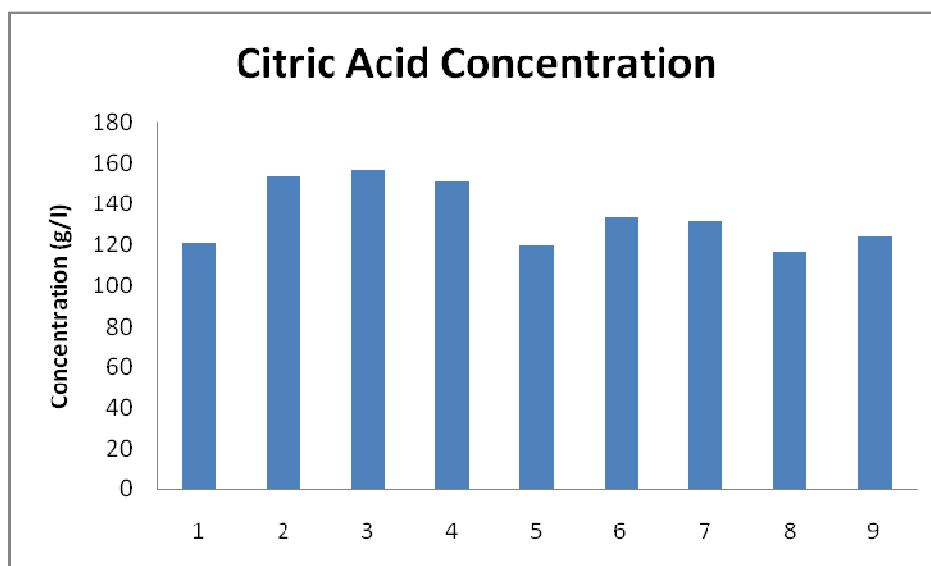
RESULTS AND DISCUSSION

After all the procedures were followed , the following result was obtained.

Table 1 Table showing the content of citric acid (in gm/lit)in each of the given sample

SL.No.	Name of Sample	Volume of sample (ml)	Volume of 1(N)NaOH (ml)	Concentration of crude citric acid (N)	Concentration of crude citric acid (M)	Concentration (gm/lit)
1.	Yam	9.7	18.4	1.896	0.6320	121.422
2.	Potato	9.9	23.8	2.404	0.8013	153.949
3.	Brinjal	9.9	24.2	2.444	0.8147	156.523
4.	Cabbage	9.8	23.1	2.357	0.7857	150.952
5.	Beet	9.8	18.3	1.867	0.6223	119.559
6.	Bean	10	20.8	2.080	0.6933	133.199
7.	Carrot	10	20.6	2.060	0.6867	131.932
8.	Lemon	9.8	17.8	1.816	0.6053	116.293
9.	Tomato	9.7	18.8	1.938	0.6460	124.112

Table 2 Representation of citric acid concentration through bar graph



From the performed experiment, it is conclusively proved that among all vegetable wastes , brinjal and potato has substantially higher yield of citric acid while lemon , breaking the conventional belief , is among the least citric acid yielding vegetable waste. This result proves beyond doubt that vegetable wastes has a great potential to be the next most valued source of citric acid and with careful attention along with finely tuned processing technology, may be one or more of them (suggestively potato , brinjal , cabbage wastes) can be commercially exploited for green production of citric acid.



Fig.2 Sample collection



Fig.3 Prepared and sealed flasks containing nutrient broth along with sample in each of them , with the antifungal agent

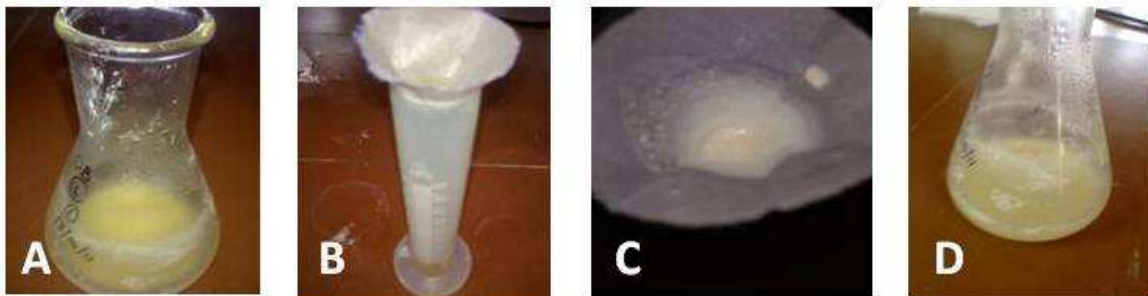


Fig.4

(Note: Fig. 4 A: Broth containing calcium citrate precipitate along with calcium salts of organic acid after boiling it in pressure cooker and addition of CaO, B and C: Separation of citrate and other salts from liquid media, D: After

treatment with sulphuric acid, appearance of CaSO₄ precipitate, which is filtered off. The supernatant is taken for titration.)



Fig.5 After titration, the colour changes from orange to blue.

CONCLUSION

In the modern Biotechnology world, waste generation and its remediation is an extremely critical issue. This research aimed at using municipal garbage wastes i.e. rotten and waste vegetables to produce an economically viable product that is the citric acid. Thus, in this manner, not only citric acid was produced from vegetative waste, but it helped to reduce and remediate municipal pollution issues to a certain extent. This has been a novel experiment, explicitly showing the potential of all the chosen sample vegetables to produce citric acid due to various organic processes and also finding out the best yielder of citric acid. Of all the modern methodologies implied for this particular extraction process, mainly involves fermentation by fungi or yeast on sugar solution. In the alternative methods, never before was this comparative analysis with an economical factor, carried out and if this method is refined further in every manner to extract very pure citric acid, not only will it be beneficial for all the food and pharmaceutical industries, but will also be instrumental in removing unwanted municipal garbage waste.

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