



A Review on Citric Acid Production and Its Applications

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Abstract

Over 100 years, citric acid remains one of the most important multifunctional organic acids produced by using traditional and modern biotechnology techniques. The citric acid has a high potential and versatile compound owing to numerous industrial applications in food and pharmaceutical industries. Globally, the citric acid production crosses more than one million tonnes and the demand increases 3-4% annually. It is important to conduct more studies on microorganisms, substrates to improve the yield for the large-scale production of citric acid to meet out the demand. In this paper, through studies are made on various microorganisms and types of fermentation for the commercial production of citric acid from different inexpensive substrates like molasses, agro – industrial wastes etc.

Keywords

Citric acid, molasses, agro – industrial wastes, biotechnology techniques.

INTRODUCTION:

Citric acid (CA), 2-hydroxypropane-1,2,3-tricarboxylic acid, is one of the familiar organic acids which is present in all citrus fruits commonly in lime, lemon and orange. In biochemistry, citric acid is one of the intermediate metabolite of Krebs cycle [1, 2]. Citric acid presents in both animal and plant kingdom. It is the valuable proof for its nontoxic nature and hence it is recognized as safe compound by WHO/FAO professional organisation for public health [3]. The citric acid is an odourless white solid chemical with the molecular formula of $C_6H_8O_7$ and with the molecular weight of 192.123 g/mol in anhydrous form and 210.14 g/mol in monohydrate form. The density of CA is 1.665g/cm³ in anhydrous form and 1.542 g/cm³ in monohydrate form. Consequently, around the world CA possess high demand and beneficial compound for daily consumptions. CA is ingenious organic acid and widely used as preservative in food industries, antioxidant & a chelating agent in numerous food processing, biochemical and medical industries [4-6]. It is stated

that 70% of the industrially produced CA were used in food and its allied industries, whereas the remaining 30% utilized in therapeutic, cosmetic and other biochemical industries [7].

HISTORICAL BACKGROUND OF CITRIC ACID:

In 1784, citric acid was first secluded by Swedish chemist Carl Wilhelm Scheele from lemon juice. In 1890, industrial production of citric acid was produced in England by treating the calcium citrate with lemon extract and diluted sulphuric acid [8]. In 1893, Wehmer revealed that some species of *citromyces* (now *penicillium*) have ability to produce significant amount of citric acid when it is grown in carbohydrate medium. However, the commercial production CA was not achieved with that strain due to contamination and time consuming process. Later, James curie in 1917 identified that *Aspergillus niger* could synthesis citric acid for the commercial purposes. In 1948, the utilization of molasses as substrate for the industrial production was successfully implemented after several studies to

minimize the substrate cost of commercial production CA [9]. In later days, the purified synthetic glucose syrup or beet or cane molasses were used as a substrate for citric acid production was developed using *A.niger*. Submerged fermentation for CA production was sturdily affected by environmental, physicochemical parameters like oxygen dispersion, mixing rate, pH, temperature, minerals & nutrients, structure of microbes, carbon and nitrogen sources and oxygen demand etc, [10]. Majorly, trace elements affect the citric acid production in submerged fermentation. Hence it becomes mandatory to use genetically modified strains which has the ability to suppress the ability of minerals or modified the raw material which has the capacity to purify the minerals [2].

MARKET DEMAND OF CITRIC ACID:

Citric acid plays a vital role in an international market because of its versatile nature and its needs in various industries. The Worldwide production of CA was 1.4 MT (Million tonnes), 1.6 MT, 1.8 MT in 2004, 2008, 2010 respectively [11, 12]. In 2016, the international market value for CA was \$2.5 billion with yearly growth rate 5% and expected to be double in 2025 [13]. The food processing industry can be reckon for 62.53% of CA international market, shadowed by medical and cosmetic industry (17.08%), detergents and cleaner industry (12.27%) and others (8.12%). Also, the augmentation out crop states that the food processing industry has higher contribution (6.03%) in CA international market from 2016 to 2021 and is shadowed by drug and cosmetic industry (5.84%). Fig I represents the application of citric acid. In 2019, China is the leading country in the production of citric acid with yearly development 4%. In 2025, CA market has the capacity to grow over 5.24% which indicates that there is a search for new CA production technologies and materials [14]. Moreover, the annual production of citric acid is near to the ground when compared to its rate of demand. Fig II represents the Market Share of citric acid.

APPLICATIONS OF CITRIC ACID:

Citric acid, due to its pleasant taste and palatability, is used as a flavouring agent in food and beverages. It is used in chemical industries as an antifoam agent. In pharmaceutical industry, it is used as a blood preservative in the form of tri sodium citrate. CA can be utilized as an agent for stabilization of fats, oils or ascorbic acid. In detergent/cleaning industry, citric acid has slowly replaced polyphosphates. Table I represents the application of citric acid in various industries.

BIOSYNTHESIS OF CITRIC ACID:

Citric acid is the primary metabolite produced in all living organisms. In mitochondria, it is mainly produced in Tri Carboxylic Acid cycle. Glucose acts as a precursor element in the citric acid production. Through, glycolysis pathway, the six carbon compound glucose breaks into two molecules of three carbon compound pyruvic acid using pyruvate dehydrogenase. By oxidative decarboxylation, pyruvate is converted to acetyl-coA. Oxaloacetate is the starting molecule of Krebs cycle. The combination of acetyl-coA and oxaloacetate forms citric acid. At the end of the cycle, oxaloacetate is regenerated [21]. Fig III represents the biosynthetic pathway of citric acid.

INDUSTRIAL PRODUCTION OF CITRIC ACID:

In ancient days, citric acid was produced by three methods i.e. fermentation, chemical synthesis and extraction from citrus fruits. For the large-scale production of citric acid, microorganisms are more reliable source when compared to plant and animal sources. Commercially, citric acid was produced by microbial fermentation with increased environmental factors and genetically improved strains [22]. The yield of citric acid differs for different substrate and fermentation process of same microorganism and the few are listed below.

Aspergillus niger

Kumar et al., (2002) stated that when the same strain of *Aspergillus niger* inoculated with bagasse and wheat bran with 65% moisture content and 29.6% sugar content in a solid-state fermentation it yields 9.4g and 6.2g of citric acid per 100g of substrate consumed respectively. He also stated that the size of the substrate also influences the production of CA. The lowest CA production was resulted in largest size (1.6 -2.0mm) and vice-versa highest yield were achieved in the substrate particle size of 0.64mm [23]. Zhangiralam et al., (2011) revealed that CA production is also affected by the culture age. The maximum yield was achieved in using 3-4 days of young and mature spores. A 72 hours culture of *A.niger* is inoculated with oil palm empty fruit branches (EFB) in Optimum conditions. The yield of citric acid is 0.4-0.41kg –EFB. Under same conditions, 48 hours culture is inoculated, the yield is below 0.3 kg-EFB. The yield amount is decreased due to the presence immature spores [24]. Addo et al., (2016) reported that the growth of microorganisms and their ability to produce metabolites were affected by incubation temperature. The *A.niger* is cultured with 15% g/l of sucrose and substrate to moisture ratio 80% for 6days at different temperature. The resultant CA production varies with respect to the

variation in temperature and yields 34g/l, 32g/l, 16g/l at 30°C, 26°C and 32°C respectively. The growth and metabolite production is decreases when the temperature decreases because of low enzyme activity and maximum yield was attained at 30°C [5].

Yarrowia lipolytica

The yeast *Yarrowia lipolytica* has the ability to produce citric acid from various biochemical metabolites. The microbial strain has the potential to grow well in various substrates such as n- Alkanes, glycerol, plant oil etc [25]. Commonly, the growth and activity of all *Y. lipolytica* strains are affected by various environmental factors such as pH, temperature, medium composition and Oxygen Transfer Rate (OTR) [17]. Patricia et al., (2015) reported that when the strain *Y. lipolytica* W29 and cultured with C/N ratio 391 and OTR level 192 in various pH conditions. At pH value 5 the yield of CA is 9.6(g/L⁻¹). When the pH is increased to 7, the yield of CA is decreased [26]. Under the same conditions, the OTR value is increased the production of CA also increased to three times. Sabra et al., (2017) stated that the type of carbon source used also affected the growth of *Y. lipolytica*. When *Y. lipolytica* is grown in two different medium with glucose and glycerol as carbon source with pH range 5.5-6.5 and temperature 28±2°C. It is founded that the growth is high in glycerol medium with specific growth rate 0.15 h⁻¹ compared to glucose medium [27].

Xioayan et al., (2015) revealed that the nitrogen source also vary the growth rate of *Y. lipolytica* and final yield of CA concentration. When corn steep liquor is used as a nitrogen source and glucose as carbon source, the biomass yield is 0.87g/g. When yeast extract is used as a N₂ source with pure glycerol as carbon source, the biomass yield is 0.64g/g. From that it is founded that corn steep liquor is a cost effective and cheap nitrogen source compared to expensive yeast extract [21].

***Candida* species**

Candida species come under the class of yeasts which is used for the production various enzymes and organic acids like lipase and acyl transferase [28]. Kim hyuk et al., (2015) reported that yeasts are potential producer of citric acid with high production rate, one of the species *Candida zeylanoidsis* not able to use sucrose. Because of these the carbon source sugarcane molasses pre-treated with invertase. Then, *candida zeylanoids* fermented with invertase treated molasses for 6 days. They produce 91g/l CA and 18.5 g/l isocitric acid [29]. Mamdouh et al., 2018 revealed that the production of citric acid using *Candida parapsilosis* NH-3 was greatly influenced by temperature. *Candida parapsilosis* NH-3 uses sugar concentration of 19.09±1.11g/l for the CA production

of 2.06±0.23g/l(216h) at 30°C. At 40°C, the production of CA is 3.55±0.02g/l with sugar consumption 3.75±1.09g/l. From this, it is concluded that the suitable temperature for CA production using *candida parapsilosis* is 40°C [30].

Apart from above mentioned microorganisms some other microbes also capable for the production of CA. Several yeast such as *Candida oleophila*, *C. guilliermondii*, *C. citroformans*, *Hansenula anomosa* and bacteria, such as *Bacillus licheniformis*, *Arthrobacter paraffinens*, and *Corynebacterium species* in various substrates. Now-a-days genetically engineered mutant strains are employed to overcome physical and chemical conditions for high productivity. Table II represents certain genetically modified strains used for CA production from various substrates.

FERMENTATION PROCESS OF CITRIC ACID:

Commercially, citric acid was produced by microbial fermentation with increased environmental factors and genetically improved strains. Currently, 736000 tonnes of CA was produced per year which was done by microbial fermentation. The microbial fermentation can be divided into three types. They are 1. Solid state fermentation 2. Surface fermentation 3. Submerged fermentation [40].

Solid State Fermentation (SSF)

Solid state fermentation is also known as Koji fermentation. In this fermentation process the microorganisms grows on the moist solid support of various organic waste material. The solid organic materials with 70% moisture serves energy and nutrients for the microbes to grow [41, 42]. The waste materials are used for this process are banana peel [43], corn stover [44], apple waste [45] etc. In SSF several strains of *A.niger* is used as a production microbes. The pH of SSF should be maintained between 4.5-6.0 and incubation temperature should be 28°C-30°C. The incubation temperature varies for different microorganisms used and completed within four days [46]. The major advantage is that it can utilize various agro based industrial waste which can reduce the pollution and protect the environment eco-friendly manner. The substrates are cheap and available throughout the year which reduces the production cost and low operation and man power. The simple technologies enable to produce high platform chemicals and leads to zero wastage disposal from the agro based industries [47]. On another hand the few drawbacks with this SSF process has very poor and heat transfer rate and microbes requires only trace amount of phosphorus and nitrogen [48].

Surface fermentation

Surface fermentation also known as liquid surface fermentation and considered as important method of CA production next to SSF. Around 20% of CA is produced annually throughout the world. The pH should be maintained between 6.0-6.5 and the temperature should be 28°C-30°C. The inoculum media must contain 100-200g/l carbon sources. Glucose syrups, beet and sugarcane molasses are used as carbon source. The microbes grow on the surface of the liquid medium. Surface process carried out in fermentation chambers or trays made of aluminium and polyurethane. The trays contain sterile nutrient medium in which inoculum is cultured as spores [49]. The sterile air is continuously supplied through the filter to avoid O₂ demand. Usually, it is the two-step process. In first step, mycelium starts to grow and forms a thick layer on the surface. In second step, mycelium produces citric acid by utilizing carbohydrates. The advantages of this process are low capital cost, low sensitive to trace elements and foam free. The disadvantages are high labour requirement, high risk of contamination, high maintenance cost [48].

Submerged fermentation

Submerged fermentation process is the most widely used fermentation process for the production of citric acid. All over the world 80% of citric acid was produced by submerged fermentation. It requires high sophisticated equipment and high energy. In this process, yield of citric acid was very high [46]. Around 700 tonnes of citric acid was produced every year by submerged fermentation. The microorganisms are dispersed throughout the nutrient medium. Usually, submerged fermentation process carried out in batch manner and continuous manner also possible. The temperature should be maintained between 28-30° C with media 100-150 g/l. and cells are exposed to homogenized uniform environmental condition [50, 51]. Stirred and airlift bioreactors are used for submerged fermentation. The merits of stirred tank bioreactor are high yield and high oxygen transfer rate. Air lift reactors are suitable for low shear sensitive microbes. The major advantages of submerged process are high yield and low risk of contamination. In this fermentation, yield is greatly affected by trace elements (Fe, Zn, Mg etc) [52].

Applications of citric acid

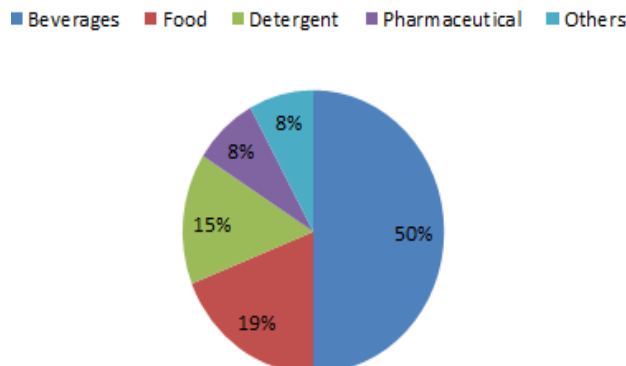


Fig I: Applications of citric acid

Market Share of Citric acid

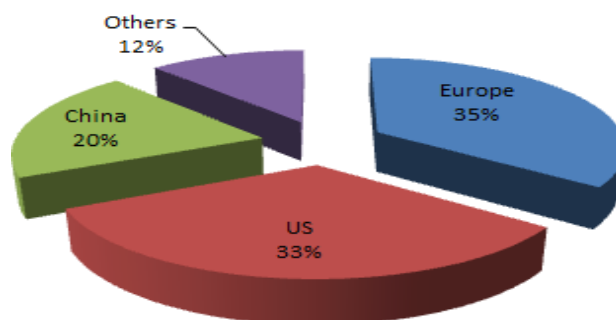


Fig II: Market share of citric acid

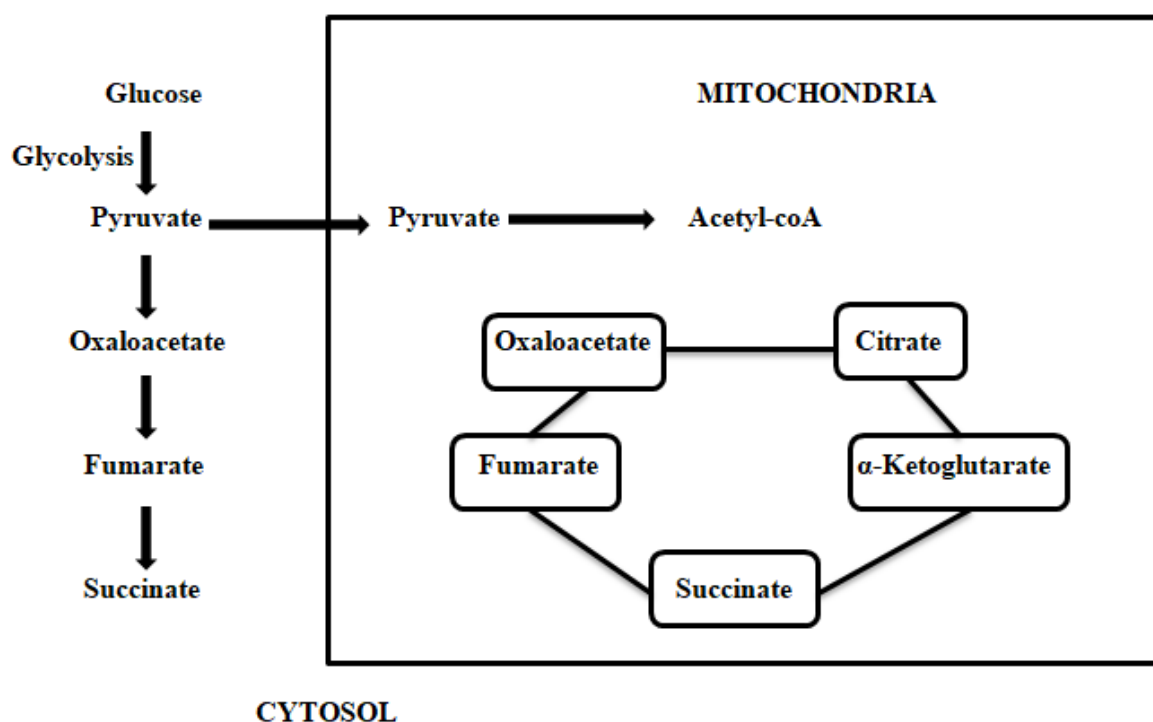


Fig III: Biosynthetic pathway of citric acid

Table I: Application of citric acid in various industries

S.No.	Industry	Uses	Reference
1.	Beverages	Prevent browning in wines. Provide tartness. Increase the antimicrobial preservatives. Adjust pH.	[15]
2.	Fats and oils	Act as antioxidant and sequestrant.	[4]
3.	Soft drinks and syrups	To stimulate natural fruit flavor.	[4]
4.	Jellies and jams	Act as gelling agent, provides tartness and flavor.	[16]
5.	Cosmetics and ceramics	Buffering agent, pH adjustment, an antioxidant.	[17]
6.	Metal cleaning	For cleaning the power station boiler.	[17]
7.	Candies	Produce dark color in candies. Reduce sucrose inversion.	[18]
8.	Dairy products	Act as emulsifier and acidifying agents in ice creams and cheese products.	[19]
9.	Frozen fruits	For inactivating oxidative enzymes and trace metals. Act as neutralizer.	[19]
10.	Pharmaceutical	Act as an element in anticancer drug, antioxidant in synthesis of vitamin, acidulant and anticoagulant. Tri sodium citrate used as blood preservatives. Ferric ammonium citrate tablets for anemia patient.	[20]

Table: II Genetically modified strains used in the CA production

S.No.	Microorganisms	Substrate/Media	Citric acid production (g/l)	Reference
1.	<i>A.niger</i> (KA88)	Corn cobs solid substrate	138.24	[5]
2.	<i>Y.lipolytica</i> W29 (ATCC 20460)	Crude glycerol	10	[26]
3.	<i>A.niger</i> (ATTC 9142)	Cocoyam starch	108	[31]

4.	<i>A.niger</i> H915-1	Corn steep liquor and corn starch	157	[32]
5.	<i>A.niger</i> ATCC12846	Wheat bran extract	162.7	[33]
6.	<i>Y.lipolytica</i> (NRRL Y-1095)	Glycerol	59	[34]
7.	<i>A.niger</i> (GMCC 5751)	Liquefied corn medium	151.67	[35]
8.	<i>A.niger</i> (FUO2)	Cassava peel malted sorghum	1.93	[36]
9.	<i>Y.lipolytic</i> (SJW-1b)	Corn steep liquor	27.5	[37]
10.	<i>A.niger</i> (FUO110)	Cassava peel malted sorghum	9.4	[36]
11.	<i>Y.lipolytic</i> (K-168)	Carrot juice-based medium	80.53	[38]
12.	<i>Candida zeylanoids</i>	Molasses	91	[29]
13.	<i>A.niger</i> (GCB 117)	Cane molasses	14.17	[39]

CONCLUSION:

Citric acid is one of the familiar organic acid with numerous applications. Globally, the production and demand of citric acid is kept increasing due to its specific application in pharmaceutical, food and cosmetic industries. The market demand, biosynthesis, microbes used for industrial production, fermentation techniques employed for the citric acid production were discussed in this paper. The concept of bio-refinery paved the way for the production of citric acid more economically and eco-friendly in many industries which ultimately leads to zero liquid discharge by employing various fermentation processes. Along with the optimized parameter condition and designed bioreactor the microbes should be genetically engineered in order to with stand against various impurities for the improved production.

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