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# Citric Acid Production: Raw Material, Microbial Production, Fermentation Strategy and Global Market: Critical Review

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## Abstract

Citric acid is an essential ingredient for the manufacture of (12) key industrial chemicals. Citric acid use is increasing steadily with a high annual growth rate as a result of the development of ever more sophisticated applications. Citric acid is widely utilized in the food and pharmaceutical industries due to its low toxicity when compared to other acidulous. Other uses for citric acid can be found in cleaning supplies and detergents. Based on information from a review of the literature, Citric acid production substrates and methods for surface fermentation, submerged fermentation, solid-state fermentation, and international market expansion are all covered in the current review study. Finally, there is still much to learn about the circumstances of the production of citric acid from raw materials, microorganisms, and fermentation techniques to achieve the best production in terms of cost and quality.

***Keywords:*** *Citric acid, Food applications, Pharmaceutical applications, Fermentation.*

## 1. Introduction

In 1893, The botanist (Carl Wehmer) discovered that the Penicillium mold could produce CA on sugar medium [1]. Carl Wehmer discovered two new fungal strains having the capacity to accumulate CA after a period of time-term, which named *Citromyces* (*Penicillium*) [2] However, because of contaminant problems and a prolonged fermentation time, industrial testing were unsuccessful. Wehmer's publications were known to Currie, a dairy scientist at the USDA, who looked at a number of other molds. Carl Wehmer observed that *A. niger*, when grown on media with a low pH, high sugar content, and mineral salts, perfectly produces citric acid. Currie's breakthrough paved the gate for the profitable industrial manufacturing of CA [3]. The most important finding was that *A. niger* thrived in pH ranges of 2.5 to 3.5 and that high sugar concentrations promoted citric acid production [4]. Since ancient times, Citric acid abounds in Iraq on Palm dates, especially dates (Zahdi) in large proportions. It is fermented by *Aspergillus niger* for 48 h under operating conditions to produce large quantities of citric acid of high quality and reasonable costs [5].

The first citric acid fermentation process was carried out in surface cultures [6]. For commercial production, some units were installed in England and Germany in the 20th century [7]. Typically, molasses is fermented by submerged microbial organisms to create citric acid for commercial use [8]. The primary method for producing citric acid worldwide is still fermentation using *Aspergillus niger* [9]. Although methods for synthesizing CA via chemical means were well-developed, microbial fermentation saw better success over time, and over time, this technology has supplanted chemical synthesis as the best option for its commercial production [10].

Despite this, there are many of issues with the introduction of submerged fermentation, choosing productive breeds that are less sensitive to trace elements. It was required to think considerably more thoroughly about the basic materials. The conditions for using inexpensive materials such starch, sugar cane molasses, beet molasses and hydrolysate starch have been optimized in many of publications [11, 12, 13, 14,]. Molasses treatment and purification methods have been developed, particularly for the removal of trace metals.

Additionally, it was discovered that a slight excess of copper ions helped produce high yields of citric acid. The demand for citric acid is increasing annually by 3.5–4.0%. In the last years, there has been a lot of interest in using agricultural wastes, such as those from the production of CA, such as those from the production of maize [15], [16], apple [17], [18], grape [19], [20], pineapple [21], [22], orange [23], [24], brewery wastes [25], [26], citrus [27], [28], and cassava [29], [30]. The market is looking for more recent, affordable, and efficient process technologies. One of the most significant commercially added-value products is citric acid, a Krebs cycle intermediary that finds use in the food processing sector (70%), the pharmaceutical industry (12%), and other industries (18%) [31]. Citric acid, also regarded as a chemical that is generally recognized as safe (GRAS), non-toxic, and pleasing to the taste. Citrus fruits like limes, lemons, grapes, oranges, and tangerines are naturally home to (CA). Citric acid is a continuously needed ingredient in the food business for usage as an acidulant, antioxidant, preservative, and emulsifier in various food items. Citric acid is so consistently employed as a standard ingredient in a wide range of food products, is a healthy molecule, and is highly demanded for daily consumption worldwide [32]. Finally, a variety of physical and chemical techniques were admitted for producing CA. However, it has been found that such conventional methods are both pricey and complex, and they are not ecological [33,34]. Additionally, when demand is strong, the annual production rate of CA is lower. In order to satisfy market demand, it is crucial for CA production to remain constant.

## 2. Properties

All aerobic organisms use citric acid, or (2-hydroxypropane-1,2,3-tricarboxylic acid), as an intermediary in their metabolic cycle [35]. The molecular weights of citric acid's anhydrous ($C_6H_8O_7$), monohydrate ($HOC(COOH)(CH_2COOH)_2.H_2O$), molecular weight (210.14) and molecular weight 192.12). White crystalline powder, respectively [36]. Monohydrate CA is sparingly soluble in ether, ethanol and sparingly soluble in both ethanol and water, CA is solid, boils at 310°C and melts 153°C at room temperature [37],[38]. It decomposes with a loss of mass above approximately 175°C ($CO_2$). It has a strong acid flavor that affects sweetness and adds a fruity tartness once dissolved in water. For these reasons, it is commonly used in the food and beverage industries to enhance fruit flavors. The acid exhibits excellent buffering capacity when combined with citrate, and its excellent metal ions chelating properties add to the physicochemical characteristics that make it ideal for food, pharmaceutical and cosmetic applications. The sheer number of these uses attests to the acid's exquisite versatility [39].

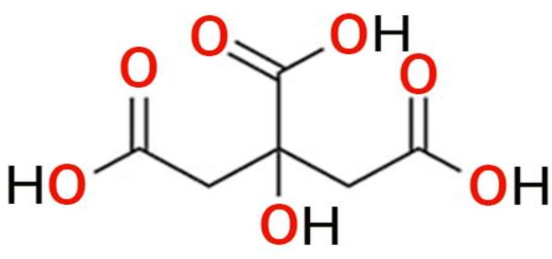


**Fig. 1. Citric acid structure.**

Microbiological conversion of acids like (Citric acid) to cellular material and other useful products is fermentation.[40],[41]. Even though it is an outdated technology, scientific advancements have kept this procedure at the forefront. The conversion process has advantages because it uses renewable resources, makes it easier to use waste for constructive purposes, and creates useful byproducts. It uses less energy and involves relatively mild environmental conditions. Additionally, it has some shortcomings, some of which are :

A-Utilization of a lot of water.

B-The garbage needs to be treated before disposal due to its high BOD.

C-Foreign microorganism contamination can lower the yield, and the technology is advanced.

## 3. Citric Acid Synthesis by Microorganisms

Microorganisms

Several microorganisms, including bacteria and fungi, have been used to produce citric acid, including *Bacillus licheniformis* [42,43], *Aspergillus niger* [44,[45], and *Yarrowia lipolytica* [46,47]. The bulk of them, however, are unable to provide commercially acceptable yields due to the fact that citric acid is a byproduct of energy metabolism and that its accumulation only increases in significant amounts under conditions of extreme imbalances. *Aspergillus niger* has continued to be the strain of choice for commercial production among those indicated since it generates more citric acid per period of time [48].

The simultaneous synthesis of isocitric acid presents a challenge in the manufacture of citric acid for yeasts. A literature review found that various microorganisms must use inexpensive basic materials as substrate to transform them into products with added value, including organic acids and other goods. Specifically, *Aspergillus niger* produces weak organic acids such as citric acid and gluconic acid [49]. The predominant black *Aspergillus* species used in this sort of manufacturing. The main reason for choosing because of what was mentioned in the literature [50]. There aren't many commercial citric acid producing industrial strains out there, and just a few may be found in foreign culture collections. By using mutagenesis and selection, citric acid-producing microorganisms have been improved. The method that has been used the most frequently is to use mutagens to cause mutations in parental strains [51]. *A. niger* mutants are employed in industrial production. UV radiation, G-radiation, and chemical mutagens are the most often using mutagens.

A single strain of bacteria can produce various amounts of citric acid depending on the fermentation technique used. Therefore, a strain that produces well in surface fermentation of solids or liquids may not always produce well in submerged fermentation. Each industrially applicable technique and raw material should thus be examined using known producer strains [52]. Spores that are supplied to the fermentation medium are utilized to inoculate the microorganisms in any method used to produce citric acid [53]. The air that is delivered into the substrate can be mixed with spores to inoculate it, or spore suspension can be used instead Glass vials with solid substrates and the ideal temperature are used to manufacture spores [54].

In terms of spore viability and citric acid synthesis, the kind of sporulation media and incubation period have an impact on the mycelia formed from *A. niger*. also, can produce citric acid from potato dextrose agar. After less than one week of spore incubation, viability increases, the ability of the spores to germinate tends to decline over time, but in certain instances, brief intervals of up to 7-8 days do not show a discernible difference from spores collected after 3 days [55]. In addition, there is a lot of interest in recent developments on the capacity of various yeast strains to make citric acid. For the past four decades, investigations on various aspects of yeasts have been the main emphasis. These studies have numerous positive correlations to fungal processes but are also infrequently utilized in business. Table (1) lists the bacteria, fungus, and yeast strains that production citric acid.

**Table 1,**
**Citric acid producing organisms.**

| Microorganism | Strains producing CA | References |
|---|---|---|
| (Bacteria) | *Arthrobacter paraffinens, Corynebacterium sp., Bacillus subtilis, Brevibacterium flavum, Corynebacterium sp Bacillus licheniformis Acetobacter xylinum* | [56]-[59] |
| (Fungi) | *A. niger, A. aculeatus, A. awamori, A. carbonarius, A. wentii,* Penicillium oxalicum *A. niger A. niger ATCC12846* | [60]-[63] |
| (Yeast) | *Saccahromicopsis lipolytica, lipolytica 1.31 Y. lipolytica NG40/UV5 Candida lipolytica* | [64]-[67] |

## 4. Different Processes for Citric Acid Production

More than 95% of the citric acid generated globally today is derived through fermentation process [68]. Citric acid is best produced by the filamentous *Aspergillus*. niger, which is also a powerful producer. The three steps that make up the production of citric acid are preparation and inoculation of the fermentation broth, fermentation, and recovery/purification of the product. Three most common methods for manufacturing citric acid are submerged fermentation (SMF), surface fermentation (SF), and solid-state fermentation (SSF), as already depicted in Fig (2). Depending on the kind of fermentation, many citric acid fermentation techniques exist.

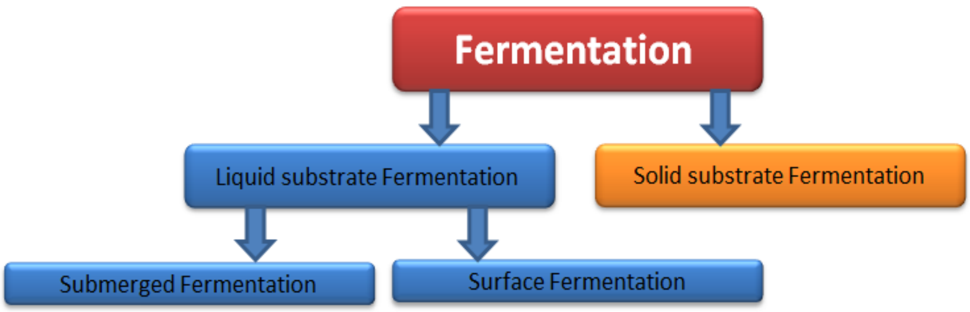


**Fig. 2. Different processes for citric acid production [68].**

From other side, As shown in Fig 3 there are many of the variables effective on productivity of citric acid include: Carbon source [69], Nitrogen and phosphate limitations [70] pH of culture medium [71], Aeration [72], Trace elements [73].

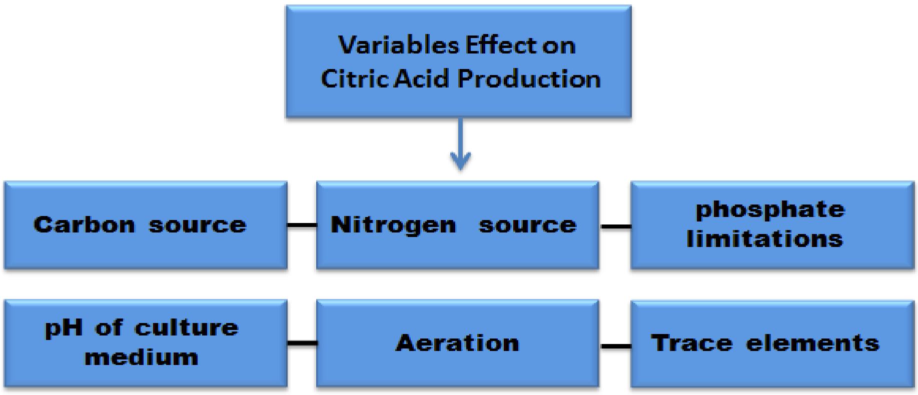


**Fig. 3. Variables effective on productivity of citric acid [69 -73].**

## 4.1 Surface fermentation

Bacteria grow on the surface of the fermentation media during surface fermentation (SF). Over the surface of the media used in the surface fermentation process used to make citric acid, *A. niger* creates a thick floating mycelial mat. Many small and medium sized industries employ surface fermentation because it is easier to operate, uses simpler equipment, and uses less energy [74], [75]. In the fermentation chambers where the process is run, many trays are arranged on shelves. The trays can be composed of high purity aluminum, steel of a certain type, or polyethylene, however steel trays offer higher citric acid yields [76]. Evaporative cooling is used to effectively manage humidity and temperature on the surface of the fermentation chambers. The chambers must always be protected and kept under aseptic conditions, especially during the first two days when spores proliferate [77]. An antibacterial filter is used to filter this air.

Aspergilli, Penicillia, Yeasts and lactic acid bacteria are the principal culprits behind the most prevalent contamination. High aeration rates are necessary to maintain temperature control and provide the microorganisms with air during fermentation, which takes 1-2 weeks to complete [78]. The contents of the tray are split into a mycelial mat and rough fermentation broth after fermentation. The mycelial mats are next cleansed to eliminate the material that had been impregnated with citric acid. Surface fermentation is less advantageous than submerged fermentation in terms of yield, labor intensity, and maintenance costs [79]. It is especially vulnerable to changes in the makeup of the media. Less energy is used during surface fermentation, and there is no foam [80].

## 4.2 Submerged fermentation

Through the SF process, the organism can develop across the liquid broth medium that is housed inside the nutrient substrate [81]. The fermentation process is carried out in bioreactors and achieved within 5-12 days [82]. After one to two days of inoculation, the organism develops into pellets that are freely suspended in the medium and have a diameter of around 0.5 cm. Therefore, an organism with a large surface area of contact can absorb nutrients and oxygen. A high-speed air flow is delivered into the vessel to increase the amount of oxygen. The air bubbles are then mixed and broken up by agitation equipment. The bacteria in the medium break down the carbon source anaerobically or partially anaerobically [83]. Superior fermentation process control and efficient usage of a variety of substrates are two of SF's primary benefits [84]. It provides lower capital, maintenance, higher production, and contamination less risks make it more suitable for citric acid production [85]. On the other hand, the disadvantage of SF is the creation of foam, which can be reduced by using antifoam agents such animal or plant fats and chambers with quantities up to one-third of the total volume of the fermenter [86].

## 4.3 Solid State Fermentation

Citric acid production process by use solid state fermentation (SSF) has garnered a lot of attention lately since it has several benefits for the production of enzymes and bulk chemicals [87,88,89]. Solid state processes require less energy, generate a lot less effluent, and offer less of a threat to the environment, which accounts for a portion of this. SSF, a fermentation process, is required for bacteria to flourish on solid surfaces in the absence of free liquid. SSF has the moisture needed for microbial growth, but it is absorbed or complexes inside the solid matrix [90]. This solid substance is typically a natural mixture made up of by products and residues from the agro-industrial and agricultural sectors, synthetic substance or a sediment. Erlenmeyer conical flasks [91], glass incubators [92], trays [93], single layer packed bed [94], packed bed column bioreactor [95], and double stage packed column bioreactor [96] are only a few examples of technique of the fermenters that have been using to produce citric acid in SSF process.

On the hand, when compared to submerged fermentation, the main benefits of solid state fermentation include, a higher production, a lower risk of pollution, lower energy, less potential for downstream processing, simplicity of operation, minimal stream generation, lower operating costs and operability under less water amounts [97]. Additionally, as the system is less sensitive to the presence of trace elements than submerged fermentation, the solid state fermentation technique enables the uncomplicated use of affordable and readily available agro-industrial substrates without the need for pre-treatment [98].

**Table 2,**
**Comparison of the production of CA using various fermentation techniques.**

| Fermentation process | Advantage | Disadvantage | References |
|---|---|---|---|
| SSF | increased production, cheaper energy and media, improved oxygen flow during installation, and less work during operation, less susceptible to the suppression of trace elements and less post-recovery, little chance of microbial pollution from waste. | Difficult parameter control and greater recovery product costs. | [99]-[102] |
| SMF | Advanced possess control systems, decrease labor costs, greater productivity and yield | Costly media, large amounts of waste water production, and sensitivity to trace metal inhibition. | [103]-[107] |
| SF | lower operating costs and energy costs. | Large heat production, space intensive, long time, and susceptible to contamination by yeasts, lactic acid bacteria, Penicillium, and fungi. | [108]-[112] |

## 5. Citric Acid Global Market

Citric acid one of the most famous natural acids, CA is a weak acid and found naturally in citrus fruits including lemons, orange and limes. It performs as a food additive and a safe preservative, helping to stabilize and preserve food products. Additionally, prevents beverage degradation and promotes emulsion preservation [113]. The global citric acid market is projected to reach USD 9 billion by 2030, growing at a Compound Annual Growth Rate (CAGR) of 5.90% for the anticipated period from 2023-2030, according to all available industry data and the development of contemporary industries in agriculture, food, and pharmaceuticals [114]. The expansion of food additives in the processed food industry has a direct impact on the CA market. During the predicted years, there is expected to be a continued high level of consumer demand for food and beverage products made using safe components. CA is being used more frequently in the pharmaceutical and cosmetics industries, which is increasing the market demand for items containing it. Citric acid helps producers meet consumer demand for safe and acceptable products while maintaining within their budgets and offering a save organic for their goods [115]. The industry is expanding due to rising consumer demand for organic food additives and changing customer preferences. Phosphate, which was formerly used, has been replaced by CA, which has increased the market. However, producers face fierce rivalry for raw materials from both outside and inside the industry, which significantly limits the growth of the citric acid market. Because of these advantages and the wide industrial applications of citric acid, the demand for it in the future will remain rapidly and growing as one of the best influential acids in the modern global market.

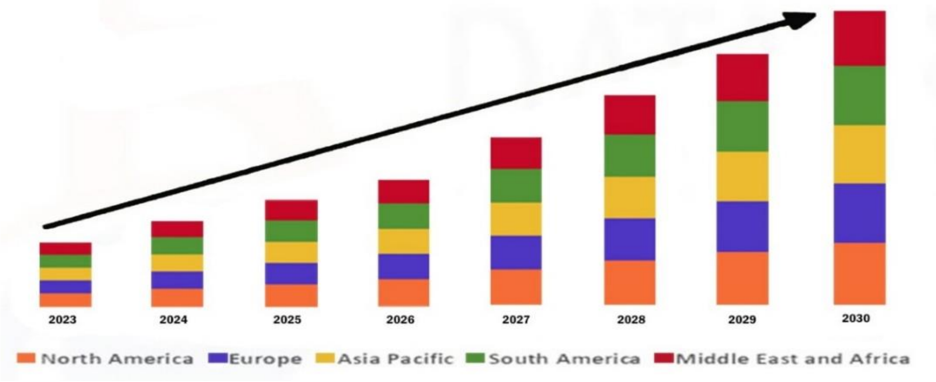


**Fig. 4. Market for citric acid anticipated to reach $9 billion globally [114].**

## 6. Conclusion

Citric acid has a wide range of applications as a versatile and secure alimentary addition across a variety of commercial sectors, including the chemical, pharmaceutical, cosmetic, and food industries. To fulfill the escalating demand for citric acid, there is an urgent need for production methods that are both affordable and environmentally friendly. It will take some time to fully comprehend the mechanics and characteristics of citric acid, and it must be generated in ways that ensure its sustainability, abundance, and excellent quality.

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# انتاج حامض الستريك: المواد الخام والإنتاج الميكروبي واستراتيجية التخمير والسوق العالمية: مراجعة نقدية

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**الخلاصة**

حامض الستريك مكون أساسي لتصنيع (12) مادة كيميائية صناعية رئيسية. يتزايد استخدام حامض الستريك بشكل مطرد بمعدل نمو سنوي مرتفع نتيجة لتطوير تطبيقات أكثر تعقيدًا من أي وقت مضى. يستخدم حامض الستريك على نطاق واسع في الصناعات الغذائية والصيدلانية نظرًا لسميته المنخفضة عند مقارنته بالحمضيات الأخرى. يمكن العثور على استخدامات أخرى لحمض الستريك في مواد التنظيف والمنظفات. استنادًا إلى المعلومات المستمدة من مراجعة الأدبيات، فإن ركائز إنتاج حامض الستريك وطرق تخمير السطح، والتخمير المغمور، والتخمير في الحالة الصلبة، وتوسيع السوق الدولية كلها مغطاة في دراسة المراجعة الحالية. أخيرًا، لا يزال هناك الكثير لنتعلمه عن ظروف إنتاج حامض الستريك من المواد الخام والكائنات الدقيقة وتقنيات التخمير لتحقيق أفضل إنتاج من حيث التكلفة والجودة.