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INDUSTRIAL PRODUCTION OF CITRIC ACID

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ABSTRACT

Citric acid, an exceptional natural product revered for its myriad uses and extraordinary properties, stands as an invaluable asset in numerous industries. This article presents a detailed overview of citric acid, shedding light on its manufacturing process and diverse applications. The manufacturing process predominantly encompasses the realm of submerged fermentation. Within the vast expanse of bioreactors, the fungus Aspergillus niger is meticulously cultivated. Paramount factors such as strain selection, growth medium composition, and the meticulous orchestration of fermentation conditions intricately shape the optimization of citric acid production. The article delves into the various crucial steps within this manufacturing process, emphasising the indispensable significance of meticulous environmental control and process mastery.

Moreover, this article takes an ambitious leap into the expansive tapestry of applications that find solace in the unparalleled versatility of citric acid. Within the food and beverage industry, citric acid reigns supreme as a preeminent acidulant, an exquisite enhancer of flavours, and used widely for preservation. However, its attributes extend far beyond the realm of gastronomy. The chelating capabilities and antioxidant prowess of citric acid position it as an invaluable ingredient in pharmaceutical formulations, personal care products, and cleansing agents. Furthermore, the influence of citric acid pervades various industrial sectors, including but not limited to metal cleansing, water treatment, and the hallowed realm of textile manufacturing. The article delves deep into these applications, illuminating the extraordinary properties that render citric acid an unparalleled and indispensable constituent across an extensive spectrum of exceptional products.

KEYWORDS: Citric acid, processing, bioprocessing, fermentation, bioreactors, manufacturing, antioxidant, molasses, oxalic acid, market.

INTRODUCTION

Citric acid is a water-soluble vitamin that is critical for a large number of regulations in the body. Citrus fruits, broccoli, kiwis, bell peppers, strawberries, and spinach are some of the richest sources of Citric acid, although it may be found in many other fruits and vegetables as well.

Citric acid, with its remarkable chemical structure and physicochemical properties, holds a significant place among organic compounds. Its molecular formula, C6H8O7, reveals a six-carbon backbone intricately adorned with three carboxylic acid groups (-COOH) and one hydroxyl group (-OH). This tricarboxylic acid, also known as 2-hydroxypropane-1,2,3-tricarboxylic acid, belongs to the distinguished class of alpha-hydroxy acids.

In terms of appearance, citric acid exists as a dazzling white crystalline powder or colourless crystals. It exhibits an exceptional solubility in water, dissolving at an approximate rate of 59 grams per 100 millilitres at 20 degrees Celsius. Ethanol also acts as a capable solvent for citric acid, while it demonstrates a limited solubility in ether. Its melting point falls within the range of 153 to 159 degrees Celsius, showcasing its solid-state nature.

With regards to density, citric acid boasts a value of approximately 1.665 grams per cubic centimetre. In aqueous solutions, it reveals its true identity as a weak acid, with a pH of around 2.2 in a 0.1 M concentration. It readily forms complexes by binding with metal ions such as calcium, magnesium, and iron. Citric acid's acidic nature emerges from its tricarboxylic structure, allowing it to donate three protons (H+) per molecule when dissolved in water. Furthermore, citric acid's stability under normal conditions makes it a reliable compound. However, it may undergo degradation under elevated temperatures or specific conditions, leading to the formation of degradation products. Its crystalline appearance, high solubility, acidic nature, chelating capabilities and many other physicochemical properties bestow upon it a multifaceted utility in various industries.

Citric acid is a potent antioxidant that helps shield cells from harm brought on by free radicals, which are unstable molecules created both naturally by the body and in reaction to environmental conditions like pollution, cigarette smoke and UV radiation. Citric acid assists in preventing oxidative stress, which has been related to a number of chronic diseases, including heart disease, cancer, and neurological disorders, by scavenging these free radicals. Citric acid tackles anti-ageing through a number of mechanisms like regulating cell signalling pathways, epigenetic regulation etc. It also boosts the immune system, aids in synthesis of collagen, promotes wound healing, absorbs iron and preserves eye health. Citric acid has been proven to relieve people of rhinovirus which causes common cold, hence Citric acid is also antiviral in nature. Citric acid can also be used for diabetes treatment, as a micronutrient during chemotherapy, in order to limit the proliferation of the cancer cell.

Geographically, the citric acid market is diversified, with North America, Europe, Asia Pacific, Latin America, and the Middle East and Africa among its major areas. Due to the existence of significant citric acid producers in nations like China, India, and Indonesia, the Asia Pacific area dominates the citric acid market. The market is booming as a result of the area's big population, quick urbanisation, growing food and beverage industry, and rising disposable incomes. During the projected period (2023-2028), the size of the global citric acid market is anticipated to increase from USD 3,481.29 million in 2023 to USD 4,268.20 million. This represents a CAGR of 4.16%.

China produces and exports more food additives than any other Asian nation, making up roughly three-quarters of the market for major ingredient categories used in the food and beverage sector. China is a prominent exporter of citric acid because it manufactures components on a huge scale and provides them at a lower price than other places. Additionally, China is a significant consumer of ingredients globally, and the country's developing pharmaceutical sector is offering potential for the citric acid market to expand. For instance, operational revenue from the l components used may vary from company to company, but a generic Citric acid supplement includes the below mentioned ingredients. The supplements sold do not contain pure Citric acid, but rather a mixture of Dextrose (17.5gm), Sucrose (14gm), Zinc Sulphate (32.5mg), and Citric acid (50mg) in order to fit the general public taste, improve the digestibility, and make it more nutritious. Some of the major companies involved in the manufacturing of Citric acid supplements are Sun Pharmaceuticals Ltd, Cipla, Lupin laboratories, Abbott health laboratories and Alkem laboratories.

Citric acid is not only used in the medical field but also in other fields like in the poultry sector for production of many different kinds of hormones such as testosterone in chicken. In birds other than chicken, Citric acid is also important for synthesis of white blood cells which indirectly improves the immune system of the bird. Citric acid was declared as an important growth regulator involved in flowering, and encourages the emergence of lateral buds. Also, it is used in synthesising highly pure, polyhedron non-agglomerated monodispersed copper powders by the reaction of $CuSO_4 \cdot 5H_2O$ and Citric acid mixture. This newfound formulation of copper powder has excellent solderability behaviour, resistance behaviour to soldering and high adhesion force due to excellent connection of the internal–terminal electrode and termination electrode–chip. Citric acid stabilises the polyethylene foam which is used in packaging. L-Citric acid, because of its structure and chemical nature, acts as a processing aid for certain foods or food ingredients, and is added to protect the quality of the food product because of its antioxidant properties.

Production of Citric acid by Submerged Fermentation

Approximately 80% of citric acid generation is accomplished through submerged fermentation, it utilises black Aspergillus, also known as *A. niger*. The process is conducted in a stainless-steel bioreactor equipped with aeration, cooling system, impellers, etc. Substrates such as beet molasses, maize starch, etc. are used as carbon sources and as a source of nitrogen, ammonia is utilised. This approach requires pretreatment of the substrate, such as nutrition addition, sanitation, etc. The culture medium is injected with A. Niger and maintained at 30 degrees Celsius. Typically, submerged fermentation is conducted in a batch bioreactor that can produce 1500 kg of citric acid and 500 kg of biomass from 2500 kg of glucose and 860 kg of oxygen. The qualities of the metal used to make fermenters, mycelium structures, and oxygen delivery systems are important factors for the production. *Candida lipolytica*, an alkane-utilising fungus, can also be used in the continuous fermentation generation of citric acid. It yields 45% more citric acid than standard production. The process for submerged fermentation is briefly explained below.

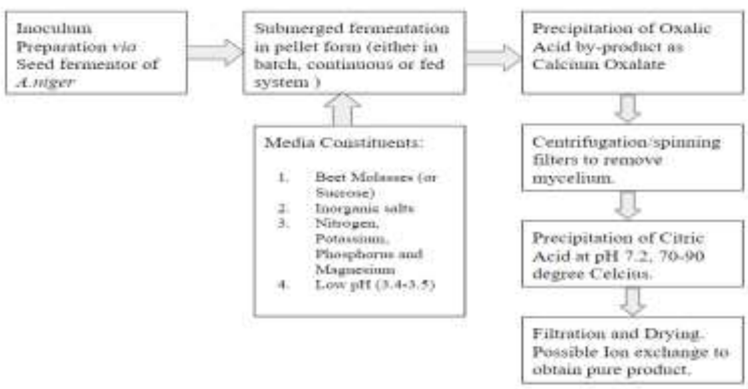

Fig1. Flowchart of citric acid manufacturing process.

1. Inoculum Production: In this method, mycelial mats known as pellets are employed as inoculum for fermentation. From a stock culture, suitable and high-yielding strains of *Aspergillus niger* are selected. A seed fermenter induces germination of the spores. In this seed fermenter, a nutrition solution containing 15% sugar from molasses is employed. Cyanide ions are introduced to the medium to stimulate the production of mycelial pellets. The production of pellets is significantly influenced by the concentration of cyanide ions in the media. If cyanide ions are in lower concentration, citric acid production is diminished.

Lower concentrations of cyanide ions encourage the production of regular mycelium rather than pellets. The spores germinate at 32 degrees Celsius and from 0.2 to 0.5 mm pellets within 24 hours. Throughout this time, the pH lowers to 4.3. These pellets are then employed as starter cultures in production fermenters.

2. Preparation of Medium: The medium used to produce citric acid must have a source of carbohydrates and inorganic salts. As a carbon source, numerous substances can be utilised. However, sucrose and beet molasses are typically employed as carbon sources. Sucrose is the best carbon source among the evaluated organic compounds. A medium containing less than 15% sucrose is reported to have a high citric acid production. When sucrose is partially replaced by fructose or glucose, citric acid output is diminished. Commercially, beet molasses (150 g/L) is widely employed as a carbon source in the manufacture of citric acid by *A. niger*. In addition to sugars, beet molasses contains an abundance of inorganic salts. Before it is utilised in the creation of the medium, it is treated with ferrocyanide or ferricyanide to eliminate these excessive inorganic salts. The inorganic salts can also be extracted by passing the beet molasses through a cation exchange resin. In addition to carbon, elements such as nitrogen, potassium, phosphorus, and magnesium are required in the media. In minimal amounts, ammonium nitrate, potassium dihydrogen phosphate or potassium monohydrogen phosphate, and magnesium sulphate are added to the medium. Higher concentrations of these components decrease the yield of citric acid and increase the output of oxalic acid. Adjusting the pH of the medium to 3.4-3.5 with hydrochloric acid is required. According to reports, a medium with a low pH facilitates less contamination, the synthesis of more citric acid, the suppression of the formation of oxalic acid, and simple sterilisation. In one litre of deionized water, salts and carbohydrates are dissolved. The medium must be sterilised at 55-103 to 69-103 Nm-2 per square inch of steam pressure for 30 minutes. Within 24 hours, granules with a diameter of 0.2-0.5 mm are generated and used for the inoculum

(10% v/v); after this time, the pH lowers to 4.3. Submerged fermentation typically takes 5 to 10 days to complete, depending on the process circumstances. It can be done in batch, continuous, or fed batch systems, with batch being the most common.

3. Fermentation Process

The majority of fermenters used for citric acid manufacturing have a capacity between 10 and 220 kilo litres. They must be made from stainless steel to prevent heavy metal leaching. Normal steel, if used in the building of fermenters, may limit the synthesis of citric acid at pH levels between 1-2. Due to their huge surface-to-volume ratio, small stainless-steel fermenters with a capacity of up to 1000 litres should be lined with plastic. Nevertheless, huge stainless-steel fermenters do not require such a plastic lining. The mycelial pellets grown in the seed tank are transferred aseptically to the fermenters and incubated at a constant temperature of 30 degrees Celsius. The structure of the mycelium that forms in the fermenter is essential to the success of the manufacturing process. If the mycelium is loose and filamentous with few branches and no chlamydospores, little citric acid is generated. The optimal amount of citric acid is produced when the mycelium is in pellet form. The iron-to-copper ratio in the media determines the type of the mycelium. In certain instances, production fermenters are directly inoculated with spores. Although *A. niger* has a low oxygen requirement, it is vulnerable to oxygen shortage. The oxygen concentration must be between 20 and 25 percent of the saturation value during the fermentation process. Short interruptions in the oxygen supply permanently halt manufacturing. During the acid generation phase, the aeration rate should be between 0.2-1 volume per minute. Low viscosity makes stirring unnecessary. Consequently, while some factories utilise stirred fermenters, airlift reactors can also be used. Foaming is an issue during submerged culture. However, it can be regulated by often adding antifoaming substances such as lard oil.

Both airlift and stirred bioreactors require a foam chamber one-third the size of the fermenter volume. There are additional mechanical antifoaming devices available. By calculating the fermentation’s sugar and citric acid concentrations on a regular basis, the fermentation’s progress is regularly checked.

4. Recovery of citric acid: The initial stage in citric acid recovery is to precipitate oxalic acid at low pH, possibly in the form of calcium oxalate, and then separate it from the media containing the mycelium using spinning filters or centrifuges. After that, at pH 7.2 and 70-90°C, citric acid is precipitated and recovered via filtration and drying. If a purer product is sought, it is dissolved in sulfuric acid, processed with charcoal or ion exchange resins, and crystallised once more as anhydrous citric acid (above 40°C) or a monohydrate (below 36.5°C).

REFERENCES

1. J. Velíšek and K. Cejpek, "Biosynthesis of food constituents: Vitamins. 2. Water-soluble vitamins: Part 1 - a review," *Czech Journal of Food Sciences*, Jan. 2008; 25(2): 49–64.
2. P. J. Accardo and M. Jaworski, "Heads Up: Plotting Children's Head Circumference," *The Journal of Pediatrics*, Jun. 2010; 156(6): 871–872.
3. Pinnell S, Yang H, Omar M, Et al. Topical L-Citric acid: percutaneous absorption studies. *Dermatol Surg*., 2001; 27: 137-142.
4. Al-Niaimi F, Chiang NYZ. Topical Citric acid and the skin: mechanisms of action and clinical applications. *J Clin Aesthet Dermatol*, 2017; 10: 14-17.
5. Ichihashi M, Ueda M, Budiyanto A, Bito T, Oka M, Fukunaga M, et al. UV-induced skin damage. Toxicology, 2003; 189: 21-39.
6. Marchese C, Maresca V, Cardinali G, Belleudi F, Ceccarelli S, Bellocci M, Et al. UVB-induced activation and internalisation of keratinocyte growth factor recep-tor. Oncogene, 2003; 22: 2422-31.
7. Pinnell S, Yang H, Omar M, Et al. Topical L-Citric acid: percutaneous absorption studies. *Dermatol Surg*, 2001; 27(2): 137-142.
8. Matsuda S, Shibayama H, Hisama M, Ohtsuki M, Iwaki M. Inhibitory effects of a novel ascorbic derivative, disodium isostearyl 2-O-L-ascorbyl phosphate on melanogenesis. *Chem Pharm Bull (Tokyo)*, 2008; 56(3): 292-297.
9. Rattanawiwatpong P, Wanitphakdeedecha R, Bumrungpert A, Maiprasert M. Anti-aging and brightening effects of a topical treatment containing vitamin C, vitamin E, and raspberry leaf cell culture extract: A split-face, randomised controlled trial. J Cosmet Dermatol, 2020; 19: 671–676.
10. Parvez S, Kang M, Chung H, Cho C, Hong M, Shin M, Et al. Survey and mechanism of skin depigmenting and lightening agents. Phytotherapy Research, 2006; 20: 921-934.
11. Kameyama KC Et al. Inhibitory effect of magnesium-l-ascorbyl-2-phosphate (VC-PMG) on melanogenesis in vitro and in vivo. Journal of the American Academy of Dermatology, 1996; 34: 29-33.

12. Grosso G, Bei R, Mistretta A, Marventano S, Calabrese G, Masuelli L, Giganti MG, Modesti A, Galvano F, Gazzolo D. Effects of Citric acid on health: a review of evidence. Front Biosci., 2013 Jun 1; 18: 1017-29.
13. Padayatty, Sebastian J.; Katz, Arie; Wang, Yaohui; Eck, Peter; Kwon, Oran; Lee, Je-Hyuk; Chen, Shenglin; Corpe, Christopher; Dutta, Anand; Dutta, Sudhir K; Levine, Mark (2003). Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. Journal of the American College of Nutrition, 22(1): 18–35.
14. Candelario-Jalil E, Akundi RS, Bhatia HS, Lieb K, Appel K, Muñoz E, Et al. Ascorbic acid enhances the inhibitory effect of aspirin on neuronal cyclooxygenase-2-mediated prostaglandin E2 production oral contraceptive pills. Journal of Neuroimmunology, 2006; 174(1-2): 39-51.
15. Aguirre R, May J. Inflammation in the vascular bed: Importance of Vitamin C. Pharmacology and Therapeutics Journal, 2008; 119(1): 96-103.
16. Chambial S, Dwivedi S, Shukla KK, John PJ, Sharma P. Vitamin C in disease prevention and cure: an overview. Indian J Clin Biochem, 2013 Oct; 28(4): 314-28. Epub 2013.
17. Hinek, A.; Kim, H.J.; Wang, Y.; Wang, A.; Mitts, T.F. Sodium L-ascorbate enhances elastic fibres deposition by fibroblasts from normal and pathologic human skin. *J. Dermatol. Sci.*, 2014; *75:* 173–182.
18. Pasonen-Seppanen, S.; Suhonen, T.M.; Kirjavainen, M.; Suihko, E.; Urtti, A.; Miettinen, M.; Hyttinen, M.; Tammi, M.; Tammi, R. Vitamin C enhances differentiation of a continuous keratinocyte cell line (REK) into epidermis with normal stratum corneum ultrastructure and functional permeability barrier. *Histochem. Cell. Biol.*, 2001; *116:* 287–297.
19. Yin, R.; Mao, S.Q.; Zhao, B.; Chong, Z.; Yang, Y.; Zhao, C.; Zhang, D.; Huang, H.; Gao, J.; Li, Z.; Et al. Ascorbic acid enhances Tet-mediated 5-methylcytosine oxidation and promotes DNA demethylation in mammals. *J. Am. Chem. Soc.*, 2013; *135:* 10396–10403.
20. Lin, J.R.; Qin, H.H.; Wu, W.Y.; He, S.J.; Xu, J.H. Vitamin C protects against UV irradiation-induced apoptosis through reactivating silenced tumour suppressor genes p21 and p16 in a Tet-dependent DNA demethylation manner in human skin cancer cells. *Cancer Biother. Radiopharm*, 2014; *29:* 257–264.
21. Prakash, A., & Baskaran, R. (2018). Acerola, an untapped functional superfruit: a review on latest frontiers. Journal of Food Science and Technology, 55(9): 3373–3384.

22. Ash, Keith, Et al. "Comparison of topical therapy for striae alba (20% glycolic acid/0.05% tretinoin versus 20% glycolic acid/10% L-ascorbic acid)." Dermatologic surgery, 1998; 24.8: 849-856.
23. Al-Niaimi, Firas, and Nicole Yi Zhen Chiang. "Topical vitamin C and the skin: mechanisms of action and clinical applications." The Journal of clinical and aesthetic dermatology, 2017; 10.7: 14.
24. Reang, J.; Sharma, P.C.; Thakur, V.K.; Majeed, J. Understanding the Therapeutic Potential of Ascorbic acid in the Battle to Overcome Cancer. *Biomolecules*, 2021; *11:* 1130.
25. KHAN, R. U., NAZ, S., NIKOUSEFAT, Z., SELVAGGI, M., LAUDADIO, V., & TUFARELLI, V. (2012). Effect of ascorbic acid in heat-stressed poultry. World's Poultry Science Journal, 68(03): 477S–490.
26. Campbell JD, Cole M, Bunditrutavorn B, Vell AT: Ascorbic acid is a potent inhibitor of various forms of T cell apoptosis. Cell Immunol, 1999; 194: 1-5. 10.1006/cimm.1999.1485.