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Biotechnological Application of Citric Acid and Kojic Acid Produced by Fungi

Waill A. Elkhateeb[1*], Dina E. El-Ghwas[1], Abdel-Nasser A. Zohri[2], Ghoson M. Daba[1]

[1]Chemistry of Natural and Microbial Products Department, Pharmaceutical Industries institute, National Research Centre, Dokki, Giza, 12622, Egypt.

[2]Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut, Egypt

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***Corresponding author:** Waill A. Elkhateeb, Chemistry of Natural and Microbial Products Department, Pharmaceutical Industries institute, National Research Centre, Dokki, Giza, 12622, Egypt.

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Abstract

Microbes are promising biotechnological tools that are used for green synthesis of numerous products. Fungi in particular are potent producer of many industrially important compounds, thanks to their prestigious enzymes machinery that allow fungi to convert substrates to desired product. Hence, this review aims to focus on properties, synthesis, and biotechnological applications of two important organic acids produced by fungi, which are kojic and citric acids.

Keywords: kojic acid; citric acid; biotechnological application

Introduction

Kojic acid: The name 'kojic acid' (which was originally known as Koji acid) was derived from "Koji", the fungus starter or inoculum used in oriental food fermentations for many centuries. Its chemical structure was previously investigated and defined as 5-hydroxy-2-hydroxymethyl-4-pyrone [1]. Kojic acid (KA) is a chelation agent produced by several species of fungi during aerobic fermentation of various substrates.

Application of Kojic acid

The most striking benefit of kojic acid is found in cosmetic and health care industries. KA has various applications in several fields such as cosmetic industry, medicine, food industry, agriculture, and chemical industry. Nowadays, KA plays a crucial role in cosmetics [2], especially skin care products which prevent exposure to UV radiation. It has been used in the production of skin whitening creams, skin protective lotions, whitening soaps, and tooth care products, and it acts as ultraviolet protector. KA suppresses hyperpigmentation in human skins by restraining the formation of melanin through the inhibition of tyrosinase formation, the enzyme that is responsible for skin pigmentation [3]. KA plays an extensive role in prevention of browning formation (speck) during processing and storage of uncooked noodles. In addition, it has also an inhibitory effect on polyphenol oxidase in different fruits and vegetables including apples, potatoes, and crustaceans [4]. At present, kojic acid is primarily used as the basic ingredient for excellent skin lightener in cosmetic creams, where it is used to block the formation of pigment by the deep cells on the skins [5]. In addition, kojic acid and its manganese and zinc complexes can potentially be used as radio protective agents, particularly against g-ray [6].

On the other hand, kojic acid has many potential industrial applications. It is the first pyrone derivative that is chemically used for analytical iron determinations in ores. Metal chelates of kojic acid have been advocated as the source materials giving the controlled release of metallic ions in curing agents or catalysts [7].

The properties of kojic acid

Kojic acid crystallises in the form of colourless, prismatic needles that sublime in vacuum without any changes. Meanwhile, the melting point of kojic acid ranges from 151ºC - 154ºC [8]. Kojic acid is soluble in water, ethanol and ethyl acetate. On the contrary, it is less soluble in ether, alcohol ether mixture, chloroform and pyridine [7]. The molecular weight of kojic acid is 142.1 [9]. Kojic acid has a maximum peak of ultraviolet absorption spectra at 280 -284 nm [10]. Kojic acid is classified as a multifunctional, reactive g-pyrone with weakly acidic properties. It is reactive at every position on the ring and a number of products which have values in industrial chemistry, such as metal chelates, pyridones, pyridines, ethers, azodyes, mannich base, and the products of cyanoethylation can be formed from kojic acid [7]. Numerous chemical reactions of kojic acid have been studied over the decades since its isolation. At carbon 5 positions, the hydroxyl group acts as a weak acid, which is capable to form salts with few metals such as sodium, zinc, copper, calcium, nickel and cadmium [11].

Synthesis of Kojic acid

The production of KA by microorganisms is alternative non-toxic and safe methods. The production of KA by aerobic fermentation of *Aspergillus* species is considered one of the best techniques used in industries. There are 58 different fungal species used for production of KA such as *Penicillium, Mucor, Aspergillus*, etc. [12]. Several *Aspergillus* species including [13], *A. tamarii* [14] and *A. parasiticus* [15], and *A. flavus* [16] have the ability to produce considerable amounts of KA in the culture medium [2]. Although several potential *Aspergillus* strains for KA production have been isolated, very little studies about the improvement of KA production by these strains through either mutation or genetic recombination techniques have been reported. Abd El-Aziz [12] reported that the mutagenesis of KA biosynthesizing genes by ultraviolet or gamma radiation caused overproduction of KA secreted by different fungi. Wan et al., [17], reported that the mutation of *A. oryzae* ATCC 22788 via chemical treatment and UV irradiation was also found to improve KA production with about 100 times higher than the parent strain. Irradiation by gamma ray may cause some mutations to the genes of cells through the DNA repair mechanisms within cells [18].

Citric acid

On the other hand, Citric acid (IUPAC) name is (2-hydroxypropane-1,2,3- tricarboxylic acid) derives its name from the Latin word *citrus*, a tree whose fruit is like the lemon. It is solid at room temperatures, melts at 153◦C, and boiling temperature is 310◦C [19]. It decomposes with loss of carbon dioxide (CO_2) above about 175◦C. It is a primary metabolic product formed in the tricarboxylic acid cycle and is found in small quantities in virtually all plants. Generally, Citric acid (CA) is an organic acid that is found in a variety of fruits such as limes, lemons, oranges, pineapples, and grapefruits. Approximately 70% of citric acid produced is used in the food and beverage industry for various purposes, 12% in pharmaceuticals and about 18% for other industrial uses [20].

Application of citric acid

Due to its pleasant taste, high water solubility, chelating and buffering properties, CA is widely used as a safe acidulant in the food, sugar, confectionery and beverages industry. In carbonated beverages, it is used to give fruit and berry flavors [21]; in the confectionery industry as flowing agent; in the wine industry to prevent turbidity of wines [22]; in candies to provide dark color and tartness; as an antioxidant synergism in fats, oils, and fat-containing foods; in sherbets as a flavor adjunct; and in ice cream as an emulsifying agent. In addition, esters of CA, such as triethyl, tributyl, and acetyltributyl, are employed as nontoxic plasticizers in plastic films that are used to protect foodstuffs. CA is used is for softening water, which makes it useful in household detergents and dishwashing cleaners or soap [23]. Also, CA has excellent metal chelating properties, and hence widely used to clean nuclear sites contaminated with radionuclides [24] and bioremediation of soils contaminated with heavy metals. It has been reported that CA in combination with rhamnolipid biosurfactants affords very excellent results in soil environmental remediation through bio-based chemical agents [24].

In the pharmaceutical industry, it is used as an antioxidant to preserve vitamins, effervescent, pH corrector, blood preservative where it prevents clotting by complexing calcium [23]. In combination with sodium citrate, acetic acid is used to prevent kidney stones [25]. It is a natural ingredient that aids in detoxification, maintaining energy levels, and supporting healthy digestion and kidney function. Also, Due to their sequestering action, such as stabilization of ascorbic acid and good buffering capacity, CA and its salts are widely used in the pharmaceutical industries as oral pharmaceutical liquids, elixirs, and suspensions to buffer and maintain the stability of active ingredients of the pharmaceutical product. Trisodium citrate is widely used as a blood preservative, CA is used in the detergent industry as a phosphate substitute [26]. Further, CA is frequently incorporated in facial packs and masks as it naturally brightens and lightens the skin tone, minimizes breakouts and oiliness, and regenerates the dead skin cells. Due to its numerous applications, the volume of CA production by fermentation is continually increasing at a high annual rate of 5% [27].

Synthesis of citric acid

At the present day most, chemical synthesis of citric acid is possible but it is not cheaper than fungal fermentation. However, a small amount of citric acid, approximately less than 1% of total world production, is still produced from citrus fruits in Mexico and South America where citrus fruits are available economically. Fungi that are reported to produce CA are *Aspergillus niger, A. awamori, A. clavatus, A. nidulans, A. fonsecaeus, A. luchensis, A. phoenicus, A. wentii, A. saitoi, A. flavus, Absidia sp., Acremonium sp., Botrytis sp., Eupenicillium sp., Mucor piriformis, Penicillium citrinum, P. janthinellu, P.luteum, P. restrictum, Talaromyces sp., Trichoderma viride,* and *Ustulina vulgaris* [28]. Several methods such as mutations, protoplast fusion, recombinant DNA technology, and gene cloning are carried out for improvement of industrially important microorganisms [29]. Among these, random mutagenesis and protoplast fusion are the simpler and commonly used techniques. The mutagenic processes involve physical, chemical, and site-directed mutagenesis for strain improvement. Improvement of strain can be also achieved by modifying the metabolism of the microorganism by inducing mutations in them through physical or chemicals mutagens [30]. The other two methods such as the single-spore technique and passage method are well-known alternative methods for the selection of improved strains [22]. Furthermore, there are basically three different types of batch fermentation process used in industry for synthesis of citric acid. These are the Japanese koji process, the liquid surface culture and the submerged fermentation process [31], but nowadays nearly all citric acid is produced by submerged culture fermentations because profitability is relatively low and thus the economics of the operation are very constricted.

On the other hand, substrate plays a very critical role to reduce cost and get optimal yield in fermentation conditions. Hence, substrate is more important for productivity and fermentation yield [32]. Increases in yield and reduction in fermentation time directly depend on the purity of substrate used. For example, *Aspergillus niger* ferment molasses, sucrose, syrups of beet or cane sugar obtained as a by-product during crystalline glucose and palm oil for CA production [32]. It has been found that cane molasses used in fermentation contains calcium, magnesium, manganese, iron, and zinc, which have a retarding effect on the synthesis of CA [33]. Also, to reduce the cost of production, agricultural waste and by-products are used in CA production. The commonly used agricultural residues including coffee husk, rice bran, wheat bran, carrot waste, cassava bagasse, banana peel, vegetable wastes, sugarcane bagasse, tapioca, cheese whey, rice straw, coconut husk, brewery wastes, decaying fruits, corn cob, orange peel, kiwifruit peel, pineapple peel, pomaces of grapes, and apples [34].

Enzymes play a very crucial role during Citric acid formation. Pentose phosphate and glycolytic pathways act as a channel by *A. niger* to metabolize glucose and accumulation of CA [35]. CA accumulation only occurred when enzymes such as aconitase (ACO), isocitrate dehydrogenase, and succinic dehydrogenase were severely inhibited during the tricarboxylic acid (TCA) cycle. However, Kubicek and Rohr [36], reported the presence of these enzymes in very less amounts throughout the CA fermentation period.

Conclusion

Fungal kingdom contains many interesting genera that are available around us and can be isolated commonly from different sources. Many promising fungal genera produce many important secondary metabolites. Fungi have been known to humans for thousands of years as they have been used in fermentation processes like wine, beer and bread making. Today, fungi are also used as alternative sources of high nutritional value proteins, enzymes and vitamins, and have numerous applications in the health food industry as food additives, conditioners and flavouring agents, for the production of microbiology media and extracts, as well as livestock feeds [37-62]. This review article has provided a brief overview of some important fungal organic acids, citric acid and kojic acid, and their applications.

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