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Improvement of Citric Acid Production by Gamma Irradiated *Aspergillus niger* Using Cane Molasses

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ABSTRACT

Citric acid is in greater demand globally as a result of both population growth and industrialization. *Aspergillus niger* is a workhorse for the production of citric acid. Cane molasses have been chosen as a novel and economically viable substrate as a result of the rising demand for citric acid and the need for substitute materials as substrates in recent years. Therefore, the present study was conducted to improve citric acid production from *A. niger* using by-product of sugar (sugarcane molasses) through gamma radiation. *Aspergillus niger* was isolated from soil and confirmed by molecular identification based on beta tubulin gene sequencing which compared with available beta tubulin gene sequencing in the NCBI database. The NCBI database showed the highest percentage of similarity being 100 with *A. niger*. The fermentation process was performed in shaking flasks using sugarcane molasses. Data revealed that significantly highest production of citric acid (31.30 ±0.17 g/L) was obtained in a medium containing (15%) substrate level. In addition, the maximum productivity of citric acid was achieved at pH 6. Spore suspensions of *A. niger* were subjected to different doses of gamma radiation. Results clarified that the maximum amount (42.82 ±0.62 g/L) of citric acid was observed at 1.0 KGy. The amount of acid produced was increased by 1.11 fold higher than the control by the addition of 3% (v/v) ethanol after 24 hour of fermentation time. Moreover, the addition of 0.15% $K_4Fe(CN)_6$ enhanced the citric acid manufacturing. Furthermore, citric acid productivity from immobilized gamma irradiated *A. niger* spores was increased by 1.2 times more than that of the free ones. Citric acid was separated from the fermentation broth by precipitation method using $CaCl_2$. In addition, FT-IR Analysis of the extracted citric acid from *A. niger* confirmed that there is no change in the main characteristic absorption bands of the standard citric acid.

1. INTRODUCTION

Citric acid is a nearly universal intermediate product of metabolism and its traces are found in virtually all plants and animals [1, 2]. It is one of the most important commercially valuable products due to its widespread use in food (70%), pharmaceuticals (12%), and other industries (18%) [3]. Citric acid can be derived from natural sources (e.g., lemon, limeand orange) or synthetic sources (e.g., chemical reaction and microbial fermentation). At present, 99% of the citric acid produced in the world is obtained by fermentation [4]. Citric acid can be produced using different raw products and agricultural wastes [5] and milling products [6].

Many organisms such as bacteria, fungi and yeast are capable to produce citric acid in their culture medium through fermentation [7]. However, *Aspergillus niger*, is superior to other microorganisms for the commercial synthesis of citric acid because of its higher end product yield when combined with fermentable sugars. It is easy to handle, can ferment various cheap raw materials, and consistently delivers high yields [8]. A number of carbon sources may be used for citric acid fermentation. For commercial reasons, the uses of molasses, sucrose or glucose syrups are favored. The use of molasses in particular is desirable because of its low cost [9].

Traditional irradiation technologies, such as ultraviolet irradiation, γ-rays, and chemical mutagenesis have been widely and frequently applied to improve the citric acid yield of *A. niger*. Hu et al. [10] reported that a high –yielding citric acid (118.9 g/L) was obtained from

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mutant *A. niger*. Immobilization of *A. niger* on calcium alginate and polyacrylamide supports has been used for the citric acid synthesis [10]. Moreover, luffa sponge, the mature dried fruits of *Luffa cylindrica* (L.) are utilized to create a natural fibrous network that is used to immobilize a variety of plants [11] and microbial cells [12]. Luffa sponge immobilization is characterized by its low cost, high porosity nature, and resistance to autoclaving, pH, and temperature fluctuations. As a result, it is often regarded as the finest carrier for industrial fermentation in poor nations [13].

Precipitation, extraction, and adsorption are three major unit processes that can be used to recover citric acid (mainly using ion-exchange resin). Precipitation is almost the widely used technique for recovering citric acid since it is adaptable to all procedures. However, it needs filtration to remove the micelles from the fungus, fermentation broth, and delayed debris [14]. Keeping in view the importance of citric acid, the current study aims at using gamma radiation to boost *A. niger's* citric acid synthesis.

2. MATERIALS AND METHODS

2.1. Collection of samples

Soil sample was collected aseptically using sterilized enclosed zipper bags with proper labeling from field planted with orange trees in Qalubiya governorate. The collected sample was carried to the laboratory on the same day and kept under normal storage conditions for microbiological studies **[**15]. Sugar cane molasses was obtained from The Sugar Refinery Factory at El- Hawamdia, Giza, Egypt. The sugarcane molasses were collected in clean, durable plastic container and stored at room temperature for further uses.

2.2. Isolation, purification and maintenance of fungal isolate

The soil suspension was prepared by dissolving 10 gram of soil sample in a 250 mL conical flask containing 90 mL sterilized distilled water and shaken thoroughly for ten minutes, and then it was left to settle for five minutes. Various dilutions such as10^{-2} to 10^{-6} were made from this stock solution in sterile distilled water under aseptic conditions. One milliliter of each dilution was then transferred to individual petri plates containing salt malt extract agar medium [16]. The petri plates were gently rotated to facilitate a uniform spreading of soil suspension. The plates were then incubated at 30ºC for 3-5 days. The appeared colonies were then picked up, sub-cultured several times for the purification and maintained on potato dextrose agar (PDA) slants at 4ºC for further studies.

2.3. Molecular identification of fungal isolate

Fungal mycelium was picked from the plates of Czapek's- Dox agar (four days age) in 2 mL sterile Eppendorff tubes. The genomic fungal DNA was extracted by freeze fracturing using liquid nitrogen as adopted by Sharma *et al*. [17] with slight modifications. Briefly, 0.2gm mycelia in Eppendorff tubes was put in liquid nitrogen for 10 min and then vigorously homogenized by a mechanical pestle. DNA extraction buffer (500µl) (200 mM Tris- HCl pH 8.0, 240 mM NaCl, 25mM EDTA and 1% SDS) was pipetted to tubes, vortex for 5 min and centrifuged for 10 min at 10000 rpm. The supernatant was gently mixed with equal volume of 1:1 v/v phenol: chloroform, for 30 min then centrifuged at 12000 rpm. The upper phase was gently withdrawn and mixed with 0.1 volume 3 M Na-acetate (pH 5.2) and 2 volume ethanol (96 %) for 60 min at -20ºC. After centrifugation, the collected DNA pellets were washed by 70% ethanol, dried and re-suspended in 100µl distilled water.

2.3.1. PCR amplification

The fungal isolate was identified based on sequencing of β-tubulin gene with the help of Solgent Company, Daejeon South Korea. Tow specific primers had been designed for identification, the first primer (ATTTCGACAGCATTTCTCAGAATTA) and the second primer (GACAGCATTTTCCAGAACGA) as recorded by Gherbawy *et al*. [18]. Amplification was conducted in a thermal cycler with an initial denaturation time of 2 min at 94ºC, followed by 30 cycles of 35s at 94ºC, 20s at 56ºC, 30s at 72ºC, and final extension at 72ºC for 1min. The purified PCR products were checked by electrophoresis on 1.4% agarose gel revealed with ethidium bromide and then visualized by gel document system.

2.3.2. Sequencing of DNA amplicons

DNA sequencing determined using a Big Dye Terminator v3.1 cycle sequencing kit (ABI) and an AB13100DNA sequencer. Sequences were further analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic relationship of the studied samples with those deposited on gene bank was constructed using clustal W software (http://www.genomejp/tools/clustalW).

2.4. Preparation of inoculum

Aspergillus niger was grown on potato dextrose agar medium. The maximum sporulation was obtained after 10 days of incubation at 30ºC. The fungal spores were scraped using a sterilized solution of saline (8g NaCl and 1mL tween 80 per liter) [19]. Then spore suspension was gathered in a sterile Erlenmeyer flask and diluted to the appropriate concentration using sterile saline ($5x10^7$ spore/mL). A standard inoculum of the spore suspension (1% v/v) was used for the fermentation medium.

2.5. Physical characteristics of the sugarcane molasses

The physical characteristics of sugarcane molasses, such as moisture content, ash measurement and pH were analyzed following standard methods [20].

2.5.1. Moisture content and ash measurement

The moisture content and ash measurement of molasses were performed by taking 10 grams of molasses sample and oven drying them in a crucible at 104°C for 30 minutes [21]. Then the results were calculated using the following equations:

$$\text{Moisture content (\%)} = \frac{A-X}{A} \times 100$$

Ash (unit) = (A-X),

Where A is the weight of molasses before burning and X is the weight of molasses after burning.

2.5.2. pH of sugarcane molasses

The pH value of molasses was measured using a pH meter model (AD1030 pH/mV/ISE) equipped with a glass electrode.

2.6. Clarification of sugarcane molasses

Sugarcane molasses was diluted by addition of water in a ratio of 1:1, acidified to pH 3.5-4.0, heated in a water bath at 90ºC for 1 hour and kept overnight to precipitate the undesirable metals. The supernatant was taken and the sediment was discarded [22].

2.7. Production of citric acid by *Aspergillus niger* from sugarcane molasses

This experiment was constructed using various dilutions of molasses in which sugar content ranged from 5 to 20 % in order to investigate the most suitable one for citric acid production**.** Each dilution was supplemented with nutrients of the modified Czapek's-Dox medium 0.3% $NaNO_3$; 0.1% $KHPO_4$; 0.05% $MgSO_4$. $7H_2O$, and 0.05% KCl (sucrose replaced with sugarcane molasses) and inoculated with (1% v/v) spore suspension of *A. niger*. Then, the inoculated flasks were incubated in a shaking incubator (150 rpm) at 30ºC for 7 days. After that, produced citric acid was estimated. This test was performed in three replicates.

2.8. Effect of different pH values of sugarcane molasses medium on citric acid production

In order to study the effect of different pH levels on the citric acid production by *A. niger*, sugarcane molasses medium was prepared and adjusted at different pH values (4.0, 5.0, 6.0 and 7.0) by adding 1N NaOH and 1N HCl. Triplet sets were made for each value. Flasks were sterilized and then inoculated with (1% v/v) spore suspension of *A.niger*. Then, flasks were incubated in a shaking incubator (150 rpm) at 30ºC for 7 days. After the incubation period, citric acid was determined.

2.9. Effect of Gamma radiation on citric acid production by *A. niger*

In order to investigate the effect of gamma radiation on the citric acid production by *A. niger*, spore suspensions ($5x10^7$spore/ mL) of *A. niger* was prepared as described before and exposed to different doses of gamma irradiation (0.00, 0.25, 0.5, 0.75, 1.00, 1.5 and 2.00KGy). The production medium was prepared and inoculated with irradiated spore suspensions separately. The inoculated flasks incubated for 7 days in a shaking incubator (150 rpm) at 30ºC. After the incubation period, the citric acid was estimated in the culture filtrates. The process of irradiation was carried out at the National Center for Radiation Research and Technology (NCRRT), located in Nasr City, Cairo, Egypt, using ^{60}Co gamma irradiation source of Indian facility with a dose rate of 1.474 kGy/h [19].

2.10. Effect of addition of different concentrations of ethanol to molasses medium on citric acid production by Gamma irradiated *A. niger*

Different concentrations of low molecular weight alcohol (ethanol) in the range between 0.0 to 5.0 % were added to the sterilized molasses medium as mentioned by Hauka *et al.* [23]. The flasks were inoculated with standard inoculum of wild and irradiated selected fungi separately and incubated for 7 days in a shaking orbital (150 rpm) at 30°C. Then acid in the filtrate was detected.

2.11. Effect of time of addition of ethanol to molasses medium on citric acid production by Gamma irradiated *A. niger*

The best concentration of ethanol that gave the highest citric acid production was added to the sterilized fermentation medium which was inoculated with the standard inoculums of gamma irradiated *A. niger* at

different times of incubation (Immediately prior to fermentation, after (24 hour, 48 hour and 72 hours), then the citric acid concentration was determined .

2.12. Effect of potassium ferrocyanide ($K_4Fe(CN)_6$) addition to molasses medium on citric acid production by Gamma irradiated *A. niger*

Sugarcane molasses medium was prepared and sterilized. Afterwards, the medium was supplemented with different concentrations of ferro cyanide (0.00-0.2%) as described by Hauka *et al*. [23]. Then the flasks were inoculated with spore suspension of irradiated *A. niger* and incubated for 7 days at 30ºC.This experiment was conducted in triplicate.

2.13. Production of citric acid by immobilized spores of Gamma irradiated *A. niger*

The sponge (which was bought from the Egyptian Company of Sponge, located in 6th Oct. City) was divided into cubes (1 cm^3) and saturated in water using continuous rinsing for a minimum of 10 times , then dried at 80ºC overnight, according to West and Strohfus [24]. The dried cubes (0.5 g) in Erlenmeyer flasks (250 mL) were autoclaved and then 50 mL sterile basal medium and 0.5 mL of irradiated spore suspension (5×10^7 spore /mL) was added. The flasks were incubated at 30°C under shaking with 150 rpm for 7 days. Irradiated spores that had not been immobilized were used as a control (free culture spores). Then the culture medium was filtered and the citric acid was calculated in the culture filtrate.

2.14. Estimation of citric acid

Citric acid estimation was carried out gravimetrically according to the method described by Marrier and Boulets [25]. One mL of the culture filtrate was transferred to the test tube. Then 1.3 mL pure pyridine was added into test tube and swirled the tube briskly prior to 5.7mL of acetic anhydride addition. The test tube was placed in a water bath at 32°C for 30 minute. The optical density was measured at 420 nm using spectrophotometer (AZZOTA SV110 digital Visible Spectrophotometer New Jersey U.S.A). The citric acid concentration of the sample was estimated from a reference (run parallel, replacing 1.0 ml of the culture filtrate with distilled water).

2.15. Recovery of citric acid from fermentation medium

Recovery of citric acid was performed using precipitation method as described by Heding and Gupta [26] with some modifications. After the completion of fermentation process, the incubated cultural broth was filtered for the separation of pellet form of fungal culture and the fermentation broth. The best agent for citrate precipitation was $CaCl_2$. In brief, equal volumes of broth and ammonium hydroxide solution were mixed and allowed to heat for 5 min. Then adequate volume of aqueous calcium chloride solution (10%) was added to the mixture and boiled to precipitate citric acid as calcium citrate. The precipitate was separated by filtration, washed by hot water and allowed to dry at 90-105ºC. Then, the precipitate was treated with 50% H_2SO_4 at 50-60ºC to precipitate insoluble calcium sulfate leaving the citric acid in solution. The solution containing the citric acid was decolorized by charcoal and it was further concentrated by evaporation.

2.16. FT-IR analysis for citric acid

The samples (dried) were examined using Fourier transforms infrared spectrophotometer (FT-IR) spectra (Thermo Nicolet iS10 FT-IR spectrophotometer). Freeze-dried samples were scanned in the range 4000-650 cm^{-1} at a resolution of 4.0 cm^{-1}.

Statistical analysis

A one-way variance analysis (ANOVA) was used to analyze the data using SPSS (version 20; IBM, Chicago, IL.USA). On the figures, the acquired data and associated standard errors of the multiple comparisons were made using Turkey's tests to evaluate the differences between groups [27], $P < 0.05$ was used to define significant differences.

3. RESULTS AND DISCUSSION

3.1. Isolation of microorganism *(A. niger)*

Citric acid can be produced by many microorganisms including fungi. A few species are known for their ability to produce high amounts of citric acid consequently, in the present investigation *A. niger* was isolated from soil on salt malt extract agar medium. The fungal isolate was identified based on preliminary morphological and cultural characteristics. Then it was subcultured on potato dextrose agar medium and stored at 4 ºC for further study.

3.2. Molecular identification

The isolated *A. niger* was confirmed by molecular identification based on beta-tubulin gene sequencing which was compared with available β- tubulin gene sequencing in the NCBI database site to ascertain their taxonomic positions(Fig. 1). The phylogenetic analysis of the fungal isolate was conducted based on neighborhood joining method. The NCBI database showed the percentage of similarity being 100% with *A. niger*.

Fig. (1): Neighbor joining tree showing phylogenetic relationship between the isolatd fungal strain and its represntative species from NCBI database

3.3. Physical characteristics of sugar cane molasses

Physical characteristics of sugarcane molasses were determined and calculated. The present study showed that the percentage of moisture content was 41.7% and the ash was calculated as 4.17% while, pH was 4.9±0.3 as shown in Table (1). These findings were in disagreement with the findings of Gasmalla *et al.* [28], they reported that the pH value of molasses was 5.8±0.35and the ash was 12.69% on wet weight basis.

Table (1): Characteristics of sugarcane molasses

Parameters	Varieties of sugarcane molasses
Moisture content (%)	41.7
Ash content (%)	4.17
pH	4.90

3.4. Production of citric acid by *A. niger* from sugarcane molasses

Dilution of molasses to a suitable sugar concentration may result in diluting unfavorable high concentrations of trace elements. In order to investigate this point, an experiment was carried out to determine the most suitable dilution of molasses that gave the highest citric acid production. The obtained results illustrated in Fig. (2) showed that the amount of citric acid and yield were significantly increased gradually by increasing molasses dilution to record the highest value (31.31±0.17g/L, 20.87±0.005%) respectively at 15%. Further increase in sugar concentration resulted in the repression of the citric acid production. It might be due to the over growth of the mycelia, which resulted in increasing viscosity of the medium and mass transfer limitations. In addition, there was statistically high significant difference in the amount of the citric acid produced at 15% as compared to the amount of the citric acid obtained in the medium containing 5,10 and 20% sugarcane molasses ($P<0.05$). Moreover, the amount of the citric acid produced by *A. niger* at 15 % was increased by 5.14 fold as compared to the citric acid obtained at 5 %. The obtained results were in agreement with results recorded by Iqbal *et al*. [7], they demonstrated that the maximum production of citric acid (14.17 g/L) was observed in the medium containing 150 g/L initial sugar concentration.

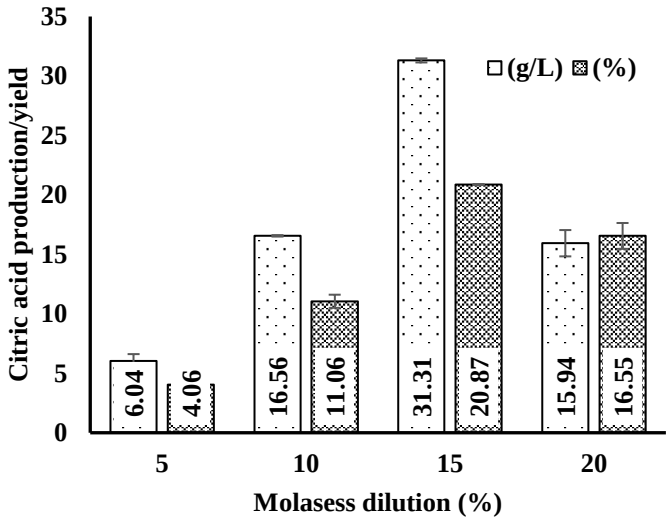

Fig. (2): Effect of different dilutions of sugarcane molasses on citric acid production by *Aspergillus niger*

3.5. Effect of different pH values of molasses medium on citric acid production

The results illustrated in Fig. (3) showed that the significantly highest citric acid production and yield (31.42±0.19 g/L, 20.95±0.02%) respectively were detected at pH 6.0. Other increase or decrease in pH value leads to a decrease in the production of citric acid. Maintaining appropriate pH value is critical for efficient citric acid production, and lowering the initial pH value reduces the citric acid production owing to low mycelium development [29, 30]. The results in the current work are in line with those of Ul-Haq *et al*. [31], they found that the highest amount of citric acid (98.92g/l) was achieved when the initial pH of fermentation medium remained constant at 6.0.

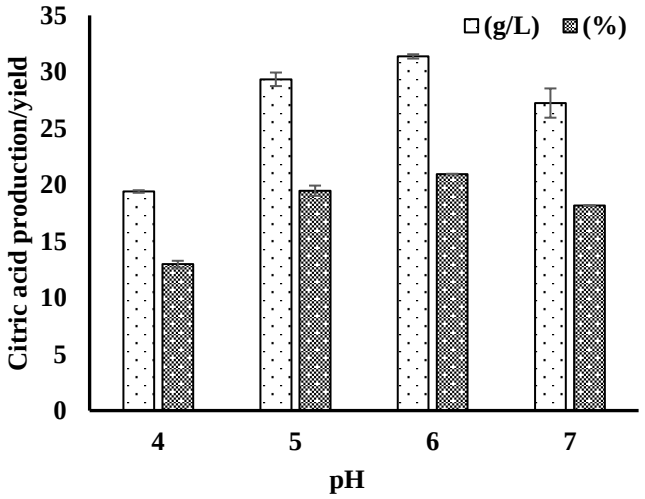

Fig. (3): Effect of different pH values of molasses medium on citric acid production by *Aspergillus niger*

3.6. Effect of Gamma radiation on citric acid production

Data presented in Fig. (4) clarified that the maximum citric acid production and yield (42.82±0.62 g/L, 28.57±0.53%) respectively were obtained at dose 1.0 KGy. The amount of the citric acid at 1.0 KGY was approximately 1.32 fold higher than control. Further increases in gamma dose result in decreasing in the citric acid production. Similarly, it was recorded that low doses of gamma radiation may induce mutants on *A. niger* and these mutants give high yield of citric acid when cultivated on molasses media [32]. Furthermore, it was reported that the low doses of gamma radiation may stimulate the microbial metabolic activities [33, 34].

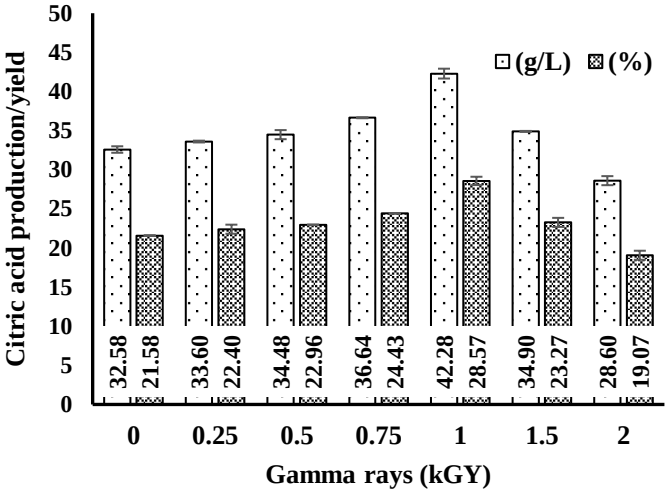

Fig. (4): Effect of different doses of gamma radiation (KGy) on citric acid production by *A. niger*

3.7. Effect of addition of different concentrations of ethanol to molasses medium on citric production

Results shown in Fig. (5) revealed that the addition of ethanol to molasses production medium stimulated the citric acid production. The significantly highest production of citric acid and yield (41.77±0.12 g/L, 27.85±0.06) respectively were achieved in molasses medium with 3% ethanol. The amount of the citric acid produced was 1.11 times higher than the control. Alcohols have been demonstrated to impact membrane permeability in microorganisms by changing the makeup of phospholipid. Other studies have found that alcohols boost citric acid synthesis through changing the attributed organization of the membrane or changes in the lipid content of the cell wall during growth and sporulation [35]. These findings were in agreement with Manonmani and Sreekantiah [36]. On the contrary, it was observed that the citric acid production from control was higher in comparison to the ethanol supplemented medium [23].

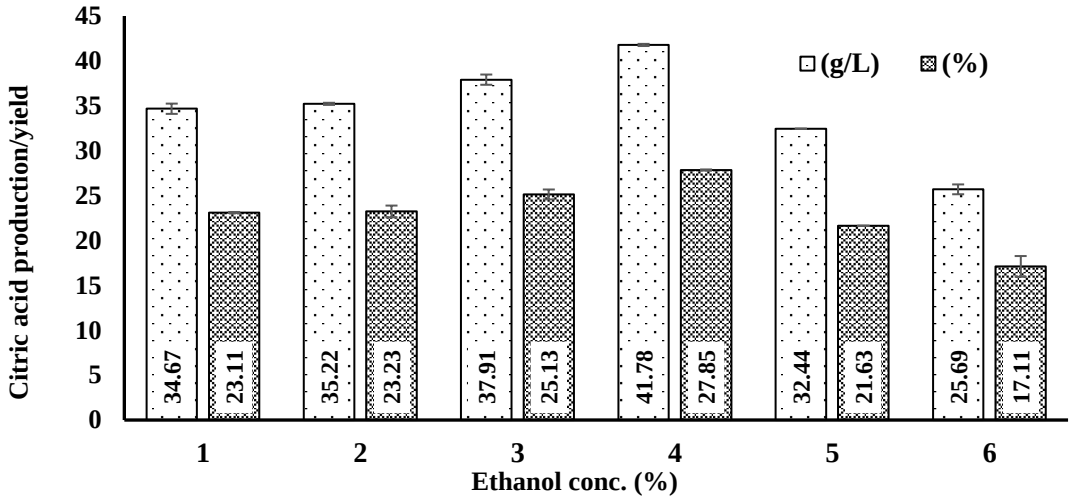

Fig. (5): Effect of addition of different ethanol concentrations (%) on citric acid production by gamma irradiated *A. niger*

3.8. Effect of ethanol addition time to molasses medium on citric production

Data presented in Fig. (6) demonstrated that the maximum citric acid production and yield (52.43±0.0 g/L, 34.95±0.03%) respectively were achieved after 24 h of ethanol addition. The quantity of the citric acid was more than that of control by 1.31 times. These data were in harmony with Ul-Haq *et al*. [37] who studied the enhancement effect of alcohols (methanol and ethanol) on the production of citric acid by a 2-deoxy D-glucose resistant culture of *Aspergillus niger* GCB-47. The greatest quantity of anhydrous citric acid (90.02 g/l) was reported 24 hours after inoculation when 1.0 percent (v/v) methanol was used as an enhancing factor. The citric acid yield was 1.96 times more than the control.

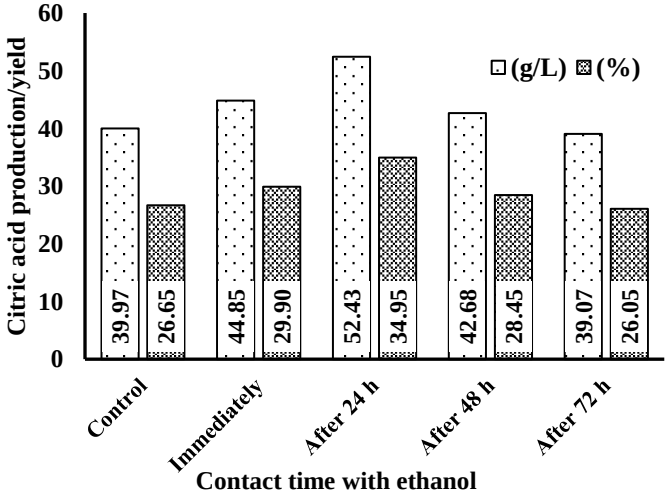

Fig. (6): Effect of time of ethanol addition on citric acid production by gamma irradiated *A. niger*

3.9. Effect of potassium ferrocyanide addition to molasses medium on citric acid production

The effect of $K_4Fe(CN)_6$ on citric acid production is illustrated Fig. (7). It was observed that the citric acid concentration and yield were significantly increased by increasing the $K_4Fe(CN)_6$ concentration from 0.05 to 0.15 %. The citric acid production from untreated molasses was low in comparison to $K_4Fe(CN)_6$ treated medium. The maximum amount of the citric acid and yield (46.71 ±0.01g/L, 31.14±0.01) were exhibited at 0.15% $K_4Fe(CN)_6$. It may be due to the fact that potassium ferrocyanide promotes the activity of some citric acid condensing enzyme by inhibiting the poisonous effect of some ions such as iron, zinc, copper, magnesium, calcium, potassium, and sodium [38]. The obtained results were in line with a previous study [39]. Moreover, these results were in harmony with Hauka *et al*. [23] who found thatthe maximum productivity of the citric acid was achieved by *A. niger* in the presence of 1.5 g/l $K_4Fe(CN)_6$ in molasses medium.

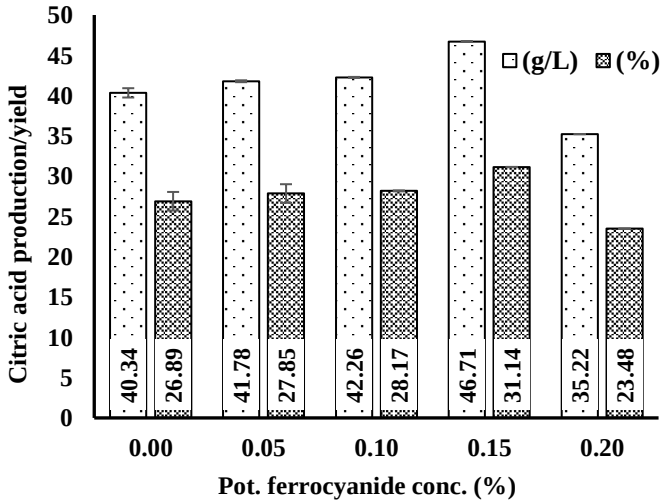

Fig. (7): Effect of different concentrations of potassium ferro cyanide on citric acid production by gamma irradiated *A. niger*

3.10. Production of citric acid by immobilized spores of radiated *A. niger*

The results illustrated in Fig. (8) revealed that the amount of the citric acid and yield produced by immobilized spores of irradiated *A. niger* were higher than free ones. Citric acid was increased by 1.21 fold higher than free one. Meleigy and Khalaf [40] used loofa sponge for immobilization of *F. moniliforme* gamma-14 cells for GA production and the authors reported that the best yield of GA (1.2 gl^{-1}) with conversion rate 2.54 % and productivity rate 8.33 $mg1^{-1}$ h^{-1} were obtained by immobilized cells after 6 days.

3.11. Stages of citric acid recovery from fermentation medium

Extraction of citric acid can be summarized in the following steps:

(i) Conversion of soluble citric acid to insoluble calcium citrate was conducted. The addition of ammonium hydroxide was followed by an acceptable volume of aqueous calcium chloride solution (10%), which resulted in the development of a calcium citrate precipitate after boiling.

(ii) Conversion of calcium citrate (insoluble) to citric acid (soluble) and calcium sulphate (insoluble) was carried out. This was accomplished by adding sulfuric acid to calcium citrate, which caused calcium sulfate to precipitate, leaving the citric acid in solution Fig. (9).

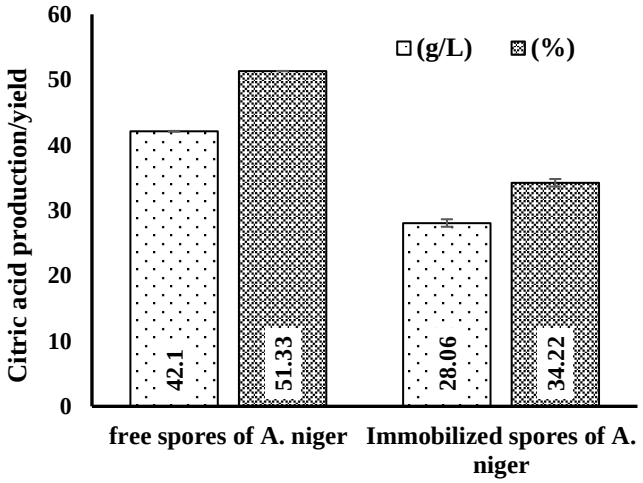

Fig. (8): Citric acid production by immobilized spores of gamma irradiated *A. niger*

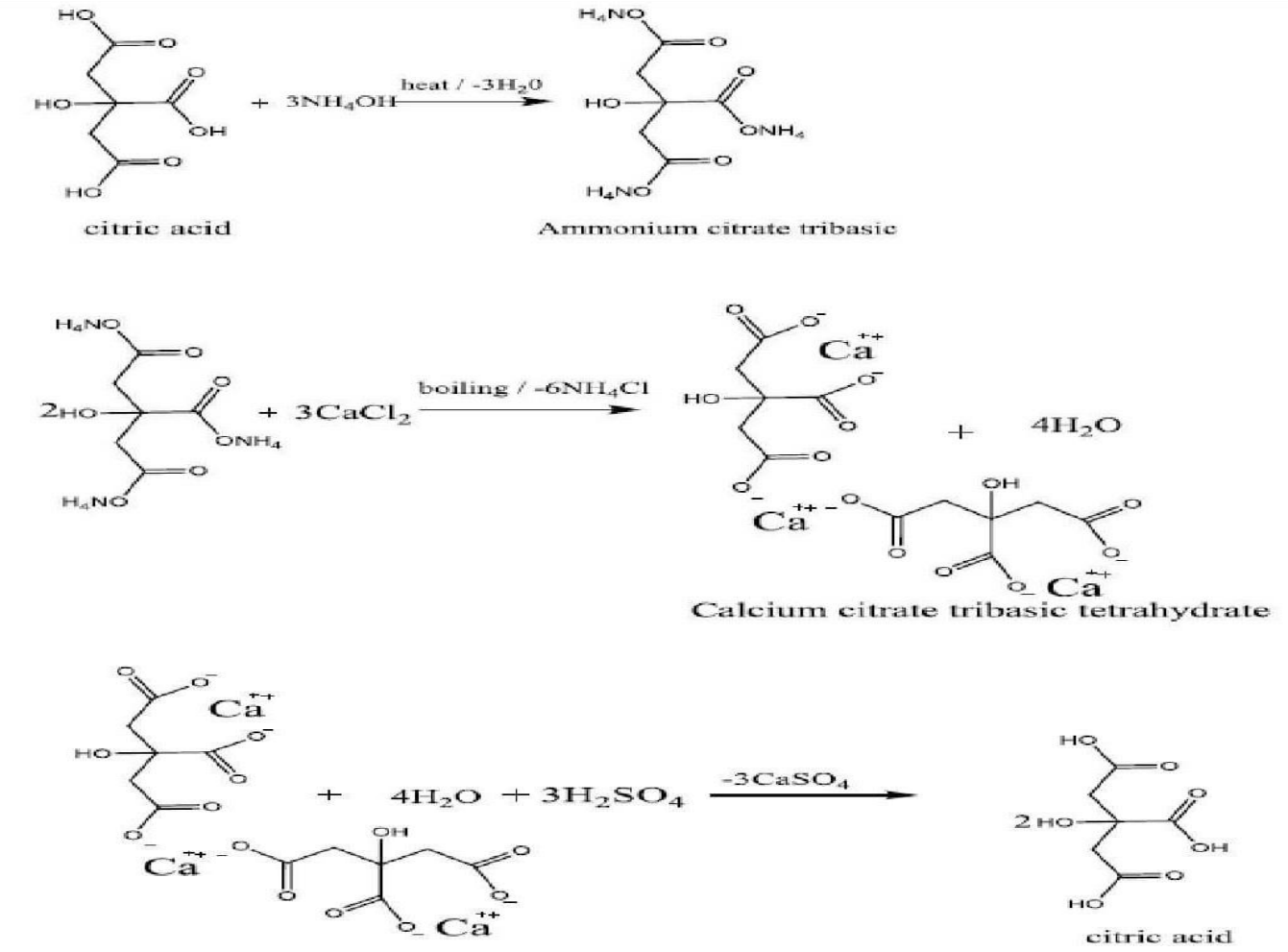

Fig. (9): Stages of citric acid recovery from fermentation medium

3.12. FT-IR analysis for citric acid

Infrared spectrum of the extracted citric acid was compared with the standard spectrum of sigma citric acid. The transmittance was carried out in the form of dried sample in the range of 400-4000 cm^{-1} using Fourier transforms infrared spectrophotometer (FT-IR Thermo Nicolet iS10 FT-IR spectrophotometer). The FT-IR spectra for the citric acid from *A. niger* in comparison with the standard citric acid is illustrated in chart(2). The main characteristic peaks in (A) curve, citric acid, are at 3469 cm^{-1} (OH group) and (C = O) stretching band at 1637cm^{-1}. In curve (B), the produced citric acid from *A. niger* culture was observed to have no change in the main characteristic absorption bands of the standard citric acid (Fig. 10).

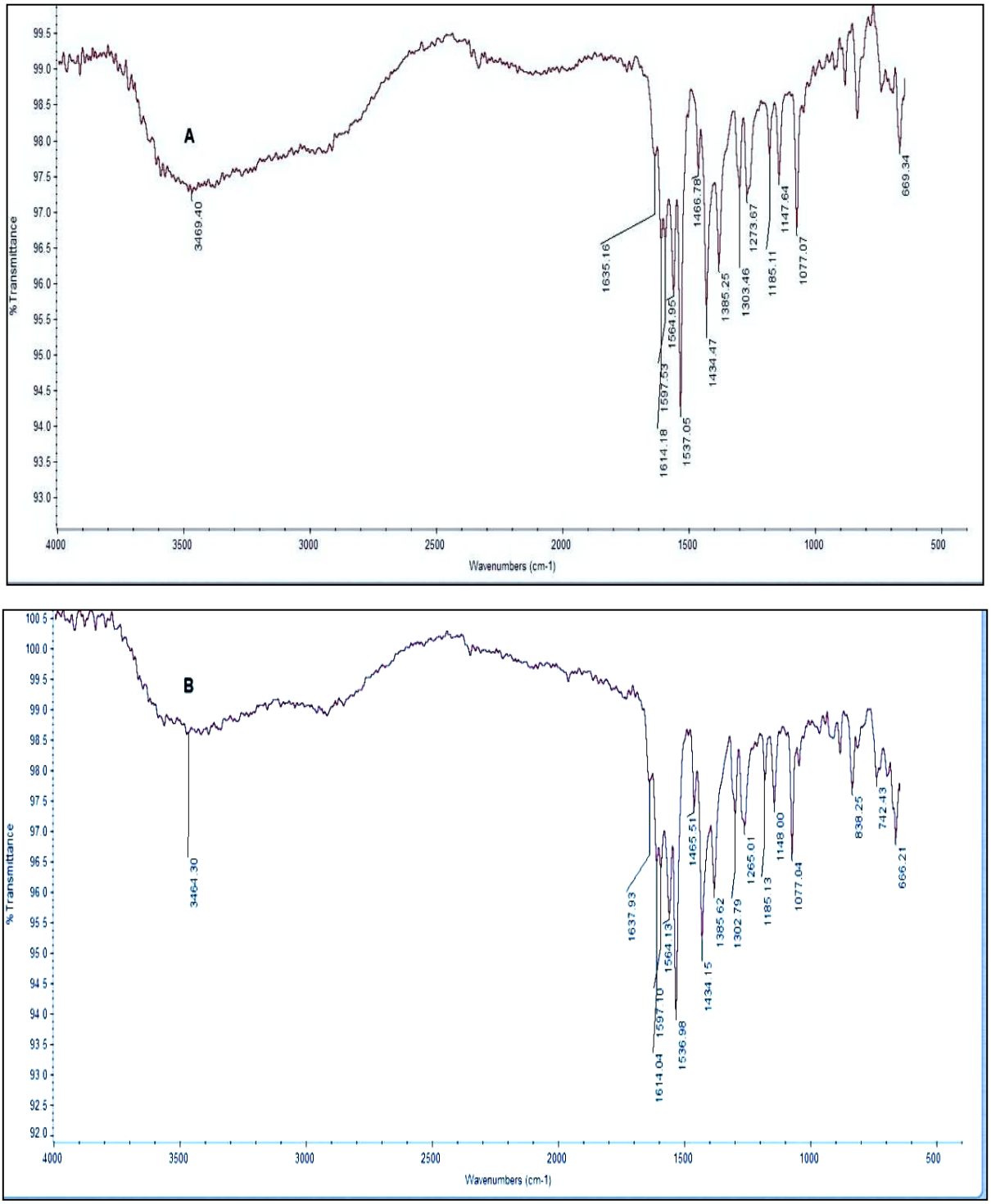

Fig. (10): FT-IR analysis of (A) standard citric acidand (B) citric acid obtained from gamma irradiated *A. niger*

CONCLUSIONS

Aspergillus niger is one of the most important fungi used in industrial microbiology, it has been employed for many years for the commercial production of citric acid. In the present study, *A. niger* was able to ferment by-product of sugar with highest citric acid production. In addition, citric acid productivity was improved after exposure to gamma irradiation. Furthermore, the amount of the citric acid produced by the immobilized spores of *A. niger* strains was higher than that of the free one. Moreover, the precipitation method using $CaCl_2$ was the best method for the citric acid recovery from fermented medium of *A. niger* .

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