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A comparative study of the ability of the fungus *Aspergillus niger* on two alternative media, wheat bran and rice husk.

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Abstract

Citric acid is one of the important organic acids in the fields of industry, agriculture, medicine, etc., and it can be obtained in many ways, the best of which is biological methods using microorganisms. The current study aimed to prepare two cheap and environmentally friendly culture media for the purpose of producing citric acid from *Aspergillus niger*. The two media, one from rice husks and the other from Wheat bran, where a comparison was made between them regarding which is more efficient in supporting the fungus *A. niger* compared to the standard medium, the best temperature and pH for production were also assessed. Eight isolates were obtained, all of which produced acid to varying degrees. No.1 was the highest and best in terms of production 3.1 gm/l, followed by isolate No. 2 with an amount of 2.5 g/l. It was observed that the amount of acid produced increased with the increase of the biomass of the fungus. The study showed variable results regarding the production of citric acid from isolates of *A. niger*, where the wheat bran medium was the best result, where the resulting concentration of acid was 5.1 gm/l, compared to 4.4 g/l for the medium of rice husks. The results showed that the best temperature was 25°C, as it gave a concentration of citric acid of 3.4 g/l, while the best pH was 6 where the concentration of citric acid produced It was 3 grams per liter.the study showed that there were no significant differences with regard to the sugars adopted in this experiment, as it gave significant similar results in terms of the amount of acid produced as well as the biomass of the fungus *Aspergillus niger*, while ammonium nitrate was the best among the nitrate sources used, with a production quantity of 4 gm/l .

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Introduction

Citric acid (lemon salt) is one of the important organic acids. It is an indispensable part of cellular metabolism, especially the citric acid cycle. It also enters the Krebs cycle and energy production [1]. It is a weak acid found in many fruits, especially oranges, lemons, pineapples, and grapefruits [2]. It is a colorless crystals and is widely used as a food preservative, prevents spoilage, and an antioxidant [3]. It is widely used in cosmetics, especially face powders, shaving creams, and detergents [4]. It helps absorb calcium and is an important material in the field of building and construction, as well as an environmentally friendly antibacterial agents [5]. It is not preferable to use it frequently because high concentrations and for a long time have side effects [6].

Because of the great demand for this acid, this acid is produced commercially using calcium salts and isocitrates, which are subjected to high heat and high pressure to obtain calcium citrate, and the latter is exposed to dilute acetic acid to obtain citric acid. This process is a dangerous process and threatens the lives of its workers. It is expensive and requires specialized and professional techniques and engineering and chemical staff [7, 8].

Another method that is safer, easier to apply, and less expensive is the use of fermentation by microorganisms. Many microorganism's species have been harnessed and were efficient in the production of citric acid, for example, the bacteria Lactobacillus Naumovozyma, Streptococcus, Trichococcus, Agrobacterium and many other species were adopted, as they gave wonderful results in this field [7, 9, 10]. As for the fungi, they were of high value in the fermentation that produced citric acid, especially Aspergillus, Penicillium, Rhizopus, Mucor, Candida, Saccharomyces, and others[5, 11-14] .This is due to the fact that fungi are characterized by a high amount of enzymes that help fermentation to occur very quickly, and they do not need complex or expensive media, but rather their requirements are simple and available[15].

A lot of studies have been conducted on determining the best conditions to produce citric acid in terms of the source of carbon, nitrogen, salts, temperature, and pH, as well as in terms of the nutrient medium [16]. Obtaining a cheap culture medium that gives abundant production of citric acid is a goal for researchers and producing companies [17]. Many alternative media have been used, including wheat bran and rice husks, and each of them gave encouraging results because they are

rich in nutrients and salts, as well as because they are available and pollutants of the environment. Therefore, their use in the manufacture of citric acid is one of the environmentally friendly production methods [18, 19].

Aims of Study

This study aimed to compare the efficiency of the wheat bran medium and rice husks in production citric acid by fermentation methods.

Material and methods

Isolation of *Aspergillus niger*

Aspergillus niger was isolated from the soil by dilution method. Soil samples were collected from different areas in the gardens of the College of Education, Al-Qadisiyah University, during January 2023, after which the samples were mixed and diluted according to what is mentioned in [20]. Then the selected concentrate was added to the potato dextrose agar (PDA) culture medium prepared for this purpose and cooled to 45 °C in conical flasks , mixed well, poured into Petri dishes and incubated at 27 °C for one week until growths appeared, where many fungi were obtained, all of which were excluded .Isolates of *A. niger* were preserved and purified by culturing them on (PDA) dishes and incubated until obtaining pure cultures. Itwas confirmed by traditional diagnostic comparison with taxonomic keys based on macroscopic, microscopic, and biochemical features [21-23].

Screening of isolates

The standard medium was used described for the purpose of screening fungal isolates [13]. The medium consisted of 15% sucrose, 0.25% ammonium nitrate (NH_4NO_3), 0.1% potassium hydrogen phosphate (KH_2PO_4) and 0.025% aqueous magnesium sulphate ($MgSO_4.7H_2O$), all of which were dissolved and mixed, then the pH was adjusted. After that, the medium was distributed in glass flasks with a capacity of 250 ml, each flask was filled with 25 ml, and sterilized by autoclaving then cooling to 25 C. Each flask was inoculated with a disc of *Aspergillus niger* fungus and incubated at 27 C for one week. After that, the amount of acid produced was estimated according to the method of [24] based on the standard curve of citric acid. The standard solution was prepared at a concentration of 10 mg/ml by dissolving 1 g of standard citric acid in a quantity of water and then complete the volume to 100 ml so that the final concentration of the acid becomes 10 mg / ml after that the standard curve was made by taking a group of ascending concentrations and with three replicates for each concentration and according to what is found in the aforementioned reference, by a spectrophotometer where the wavelength and standard curve were calculated and plotted for each isolate[25].

Also, the dry weight of biomass was test by taking the mycelium and drying it in oven with 55 ℃ overnight then it measured by sensitive balance [26].

Preparation of rice husks medium

Medium preparation of rice husk quantities of rice husk was brought from one of the mills in the city of Diwaniyah, and the method mentioned in [12].

In preparing the culture medium, the husks were washed well with plain water, then with distilled water, then dried in an electric oven at 55 degrees for 24 hours. Then we took 100 gm of rice husks and placed them in a glass beaker with a capacity of 500 ml. Then the mixture was boiled at 100 m for 8 hours, then left to cool and filtered. Using Whatman No. 1 filter papers, discarding the sediment, and taking the filtrate. Use the filtrate to dissolve the components of the citric acid production medium mentioned in the previous paragraph, adjust the pH to 3.5, and sterilize it by autoclave. Then, after cooling, distribute the medium in 250 ml beakers, 150 ml for each beaker, and inoculate each beaker with a disks 7 mm of a pure colony of the fungus *Aspergillus niger*, after which the flasks were incubated at 30 degrees for one week with continuous shaking [12].

Preparation of wheat bran medium

It was prepared by following a method by [27] bringing a quantity of bran from a bakery in Diwaniyah, 100 gm of which was mixed with 500 ml of water in a glass beaker, then boiled at 100 m for 8 hours, after which the mixture was cooled and filtered using Whatman No. 1 filter paper, and the filtrate was taken and used as a solvent for the components of the standard culture medium for the production of citric acid, according to the method mentioned in the previous paragraph[28].

After we had two media, one from rice husks and the other from bran, and after inoculating them with Aspergillus niger fungus and incubating them in a shaking incubator for a week, the curve was made for citric acid, and the ability of the two media to produce was compared in comparison with the standard curve for the purpose of comparison. The control treatment was cultures of *Aspergillus niger* fungus on standard culture medium mentioned in [28].

Effect of temperature and pH on citric acid production

To determining the optimal conditions for the production of citric acid, three temperatures were used: 20, 25, and 30 °C, and three pH were 5, 6, and 7. Where liquid tissue cultures were prepared from the standard medium for acid production, the medium was distributed in 250 ml class flasks, and then inoculated with the *A. niger*, using the method mentioned in[29], after which the incubation was carried out at the above temperatures, as well as at the above pH. .

Effect of carbon and nitrogen source on citric acid production

To detect the best carbon and nitrogen source that supports the production of citric acid from the fungus *A. niger*, a group of chemical compounds were selected, and they included ammonium nitrate (NH_4NO_3), ammonium carbonate(NH4)2CO3, and potassium nitrate (KNO3) as a nitrogen source [26].

While several compounds were used as a carbon source, including sucrose(C12H 22O11), glucose (C6H12O6) , and dextrose (C6H14O7), the standard culture medium mentioned in the above paragraph was prepared using the same method and the same cultivation conditions, except that each time the substance to be tested is added for its effect on the citric acid production [24].

Results and Discussion

Screening of *A.niger* isolate in production of citric acid

The current study involved isolating a few soil fungi, and all isolated species were neglected, and *Aspergillus niger* fungus was kept because it is preferred in the production of citric acid.

Eight isolates were obtained, all of which produced acid to varying degrees. The number 1 isolate is the highest and best in terms of production 3.1 g/l, followed by isolate No. 2 with an amount of 2.5 g/l, while the rest of the isolates came in quantities of lesser amounts of 2.2, 1.9, 1, 1, 0.8 and 0.2 g/lrespectably. It was observed that the amount of acid produced increased with the increase of the biomass of the fungus.

The genus Aspergillus is one of the most soil fungi producing citric acid, due to its great ability to grow rapidly and spread in all environments, even harsh ones. It does not need complex nutritional requirements, and this is due to its metabolic system and its ability to produce a large number and mount of enzymes, especially hydrolysis enzymes. Widespread, restorative, produces many spores that are carried by air currents to reach all places, and as soon as they get moisture, they germinate to give a colony of fungus, which in turn produces various metabolites, including citric acid. The difference in the amount of production is due to the genetic content because the acid production process citric acid is genetically and genetically controlled, and the higher the gene expression, the higher the production [4, 11, 30].

The relationship between the production of acid and the biomass of the fungus is a direct relationship, and this is self-evident because the greater the number of cells, the higher the number of pregnancies, given that the cell is like a factory to produce citric acid [30].

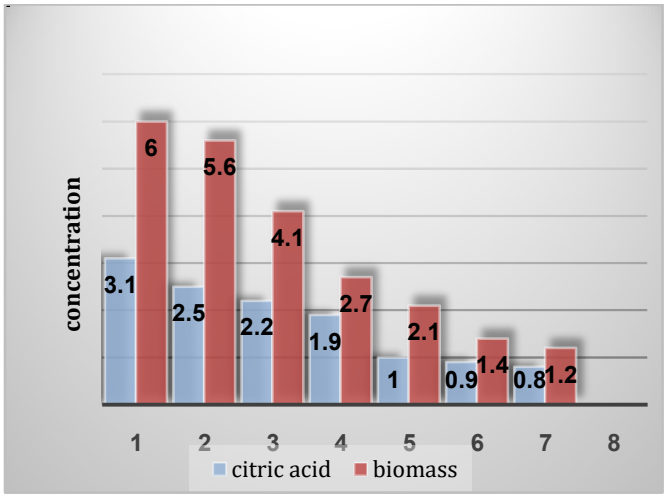

Figure (1) *A. niger* isolates activity in production of citric acid and them biomass.

Comparing between wheat bran and rice husks media in production of citric acid by *A. niger*

The study showed variable results with regard to the production of citric acid from isolates of *A. niger*, where the wheat bran medium was the best result, where the resulting concentration of acid was 5.1g/l, compared to 4.4 g/lfor the medium of rice husks, and the standard medium was the lowest concentration of acid 3.3g/l for isolate No. 1 .The No. 2 isolate gave concentrations are 4.5, 3.9 and 2.1g/l, respectively, while isolate 3 gave an acid concentration of 4.6, 3.1, and 2.6g/l respectively.

Wheat bran is a substance very rich in sugars, proteins, salts, and fats, which makes it the best in supporting the growth of fungus and increasing its biomass, which leads to an increase in the number of cells and thus an increase in the concentration of citric acid. This also applies to rice husks, but it seems that it contains fewer nutrients compared to wheat bran [31].

As for the reason for the superiority of the two-alternative media (bran and husk) over the standard medium, this is self-evident because we replaced the water (without nutrients) in which the contents of the medium are dissolved. We replaced it with wheat filtrate and rice filtrate, which enriches the nutrient medium and increases biomass, thus increasing the amount and concentration of acid produced [32].

Table (1) effect of media type on citric acid production by *A. niger*.

isolates	Citric acid (g/l)			
	Wheat bran	Rice husks	Stander medium	
1	5.1	4.4	3.3	LSD= 0.4
2	4.5	3.9	2.1	
3	4.6	3.1	2.6	
4	2.8	2	1.9	
5	2.1	1.7	1.4	
6	1	1.1	1.5	
7	1.3	0.9	1	
8	0.9	0.7	0.3	

Effect of temperature and pH on citric acid production

Table 2 shows the response of *A. niger* fungus to different levels of temperature and pH, the results showed that the best temperature was 25 ° C.The isolates it gave a concentration of citric acid of 3.4, 3.2.8, 2.2, 1.8, 1.5,2 g/l and 1.1g / l respectively, while the best pH was 6 where the concentration of citric acid produced It is 3,2.7, 2.2,2,1.7,1.4,2 and 1.1g\l.

Studies indicate the direct relationship between the biomass of the fungus and the metabolic materials it produces [12]. The more the surrounding conditions help the fungus to grow and expand on the nutrient medium, the higher the production rate will be [33]. On the contrary, the metabolic reactions are the most intense, on the one hand, and on the other hand, there are isolates that have a genetic predisposition and high gene expression to produce citric acid, which makes them produce citric acid in large quantities [34].

Table (2) effect of temperature and pH on citric acid production (gm/l)

isolates	pH			Temperature (°C		
1	2.1	3	2.7	3	3.4	2.9
2	1.9	2.7	2.5	2.6	3.0	2.2
3	1.5	2.1	2.1	2.2	2.8	2.3
4	1.3	2	1.6	1.8	2.2	1.5
5	1.1	1.7	1.3	0.7	1.8	1.7
6	0.7	1.4	0.5	1	1.5	2
7	0.5	1.1	0.3	0.5	2	0.8
8	0.3	0.6	0.3	0.2	1.1	0.4

LSD=2.2

Effect of carbon and nitrate sources

With regard to the effect of carbon sources, the study showed that there were no significant differences with regard to the sugars adopted in this experiment, as it gave significant similar results in terms of the amount of acid produced as well as the biomass of the fungus *Aspergillus niger*, while ammonium

nitrate was the best among the nitrate sources used, with a production quantity of 4gm/l followed by ammonium sulfate at 3.4 gm\l of time, then potassium nitrate 2.7 gm\l with biomass reach to 5.2 ,3.3 and 4 gm respectively. These results can be explained by cellular metabolism interactions, where the higher the substance has a high affinity towards the cellular components, the more it becomes polarized by the fungus[35]. This can also be explained by the chemical composition of the chemical, its molecular weight, its ability to dissolve in water, the type of chemical bonds, as well as the molecular weight [36]. The substance involved in the structural reactions of the cell causes the growth of the cell and the expansion of the mycelium, and the cell becomes working at its highest energy, and here the process of producing citric acid will increase, especially if the surrounding environmental conditions are appropriate and when the nitrogen and carbon sources involved in the production process are available and easy to digest and convert into simple materials [37].

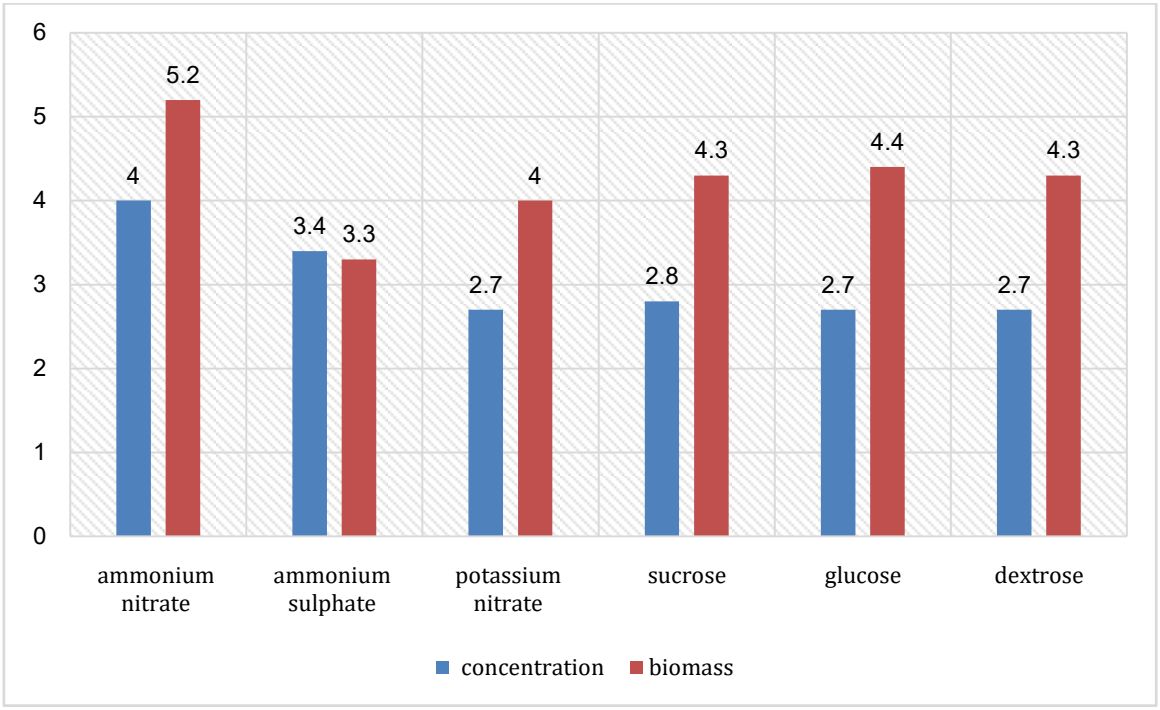

Figure (2) Effect of carbon and nitrogen sources citric acid production by *A.niger*.

Conclusion

- There are many isolate of *A.niger* capable to produce citric acid some of them lower concentration other high.
- The wheat bran medium gave the best results in production of citric acid by *A.niger*, dew for the nutrient material located in it.
- The temperature degree 25 and pH 6 were the best in production of citric acid by *A.niger* because they support the biomass of this fungus.
- Ammonium nitrate as nitrogen source was the best in supporting citric acid production, while all type of used sugars gave the same results.

Conflict of Interest

Authors are declared No Conflict of Interest

Acknowledgement

Not Applicable

Author Contribution

All Authors Contributed equally

Ethical Considerations

Not Applicable

Inform Consent

Not Applicable

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