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Original article

## Optimization of citric acid production by *Aspergillus niger* using two downgraded Algerian date varieties

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### ABSTRACT

In the present work, the GHARS and the MECH DEGLA downgraded date varieties were used in a fermentation medium in order to produce citric acid by the *Aspergillus niger*. The biochemical characteristics of the dates were investigated, along with the chemical and physical characteristics of the solutions of both samples. The analyzed parameters included the moisture and sugar content, the ash residual, the pH values, and the electrical conductivity. The effect of the following fermentation parameters was studied: initial pH, temperature, incubation period, and methanol. For the GHARS and MECH DEGLA date varieties respectively, the ash residual measured at 1.90% and 2.47%. For each date variety, the moisture and total sugars were measured at 11.59% and 85%, for the GHARS, and 12.82% and 80.47% for the MECH DEGLA. Citric acid production using either of the two varieties of dates showed a high yield in a short time.

The obtained results showed that the highest production of citric acid by both medium of dates was achieved at the initial pH value of 3.0, temperature 30 °C, and an incubation period of 8 days. Also, the maximum amount of citric acid was produced when both mediums contained 4% of methanol. Both varieties of dates showed a good yield for the citric acid and can be used as a culture medium since they are economic and ensure good growth for the *Aspergillus niger*.

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## 1. Introduction

*Phoenix dactylifera* L., commonly known as the date palm, is a primeval plant that has been cultivated for its edible fruit in the desert oases of the Arab world for centuries. The fruits are a rich source of carbohydrates, dietary fibers, and certain essential vitamins and minerals. The date pits are also an excellent source of

dietary fiber and contain considerable amounts of minerals, lipids, and protein (Baliga et al., 2011).

Algeria is the third largest producer of dates in the world with 934,377 T (FAOSTAT, 2014) and about 940 cultivars (Bedjaoui and Benbouza, 2020). Being a large country, Algeria cultivates several varieties of dates, including the DEGLA BEIDA, TALMINE, HAMRAY, HMIRA, MECH DEGLA, DELGET NOUR, GHARS, and so forth (Bouguedoura et al., 2015).

Biotechnology is an interdisciplinary technology field that is largely oriented towards the industrial applications of microorganisms for the conversion of waste to useful products. The number of substances excreted by microorganisms has yet to be fully measured. The secretions are classified as either simple compounds (eg. lower alcohols, acids, etc.) and complex compounds (eg. natural products and cellulose), or as preliminary products and compounds evolving from secondary metabolism (Zentou et al., 2019). Several fermentation procedures are used for the large-scale pro-

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duction of organic chemicals and high-energy fuels from renewable sources (Angumeenal and Venkappayya, 2013).

Citric acid (2-hydroxy-propane-1,2,3-tricarboxylic acid) derives its name from the Latin word citrus from the citrus tree, the fruit of which resembles a lemon. The acid was first isolated from lemon juice in 1784 by Carl Scheele, a Swedish chemist (1742–1786) (Yigitoglu, 1992; Show et al., 2015). Citric acid is a tricarboxylic acid with a molecular weight of 210.14 Da. In view of its three carboxylic acid functional groups, it has three  $pK_a$  values at pH 3.1, 4.7, and 6.4. Citric acid is a nearly universal intermediate product of metabolism and its traces are found in virtually all plants and animals (Papagianni, 2007; Zentou et al., 2021). It is one of the most important commercially valuable products due to its widespread use in food (70%), pharmaceuticals (12%), and others (18%) industries (Dhillon et al., 2011).

Citric acid can be derived from natural sources (e.g., lemon, lime and 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 (Kuforiji et al., 2010).

The formation of citric acid as a byproduct, while using leftover otherwise unusable cheap substrates and biodegradable waste of many industries, will reduce the related waste disposal problems (Sawant et al., 2018). It will also reduce the dependency of the industry on other sources of the citric acid production. The biomass generated during the fermentation process to produce the citric acid can be effectively utilized for biogas production and in fertilizer preparations. Thus, the industry would be benefitted ecologically and economically (Sawant et al., 2018).

A large number of microorganisms, including bacteria, fungi and yeasts, have been employed to produce citric acid. However, *Aspergillus niger*, a commonly found fungus, 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 (Show et al., 2015).

It has been shown that citric acid production by *Aspergillus niger* is markedly influenced by a number of environmental conditions and some trace elements. To develop a process for the maximum production of citric acid, standardization of the media to be used and the fermentation conditions are crucial (Bekir et al., 2009). It is well established that nutritional conditions strongly affect the production of citric acid (Ali et al., 2012).

In countries like Algeria, where dates are mass-cultivated for commercial use and as an economical source of carbohydrates both for human and animal consumption, we can have access to them also as a substrate for the commercial production of citric acid.

In this study, we used two varieties of downgraded Algerian dates, the GHARS and the MECH DEGLA, for the production of the citric acid. The focus was on optimizing the conditions during fermentation, in order to determine the best parameters, while not wasting resources or detrimentally elongating the process.

## 2. Material and methods

### 2.1. Preparation of inoculum

In this study, we used the lyophilized spores' fungus strain of the genus *Aspergillus*: *Aspergillus niger* ATCC 16888™ derived from the International Strain Bank, American Type Culture collection (Product Format SWIK-STIK Ref: 0245P) and treated it according to the procedure provided by the ATCC. The strain provided from ATCC was chosen as the best producer of citric acid (Kaouthar et al., 2020). The suspension was prepared by cultivating *Aspergillus niger* on P.D.A (Potato-Dextrose-Agar) in petri dishes at 30 °C, the original recommended temperature. After four days, spores

appeared on the surface. They were recovered in sterile distilled water and were counted using the Malassez cell-counting chamber.

The fungal suspension of conidia was then diluted to a concentration of  $3 \times 10^8$  spores/mL (Alam et al., 2010).

### 2.2. Medium preparation and fermentation conditions

The substrates used were the two varieties of Algerian dates, the GHARS and the MECH DEGLA dates from the region of Tolga in Biskra, Algeria.

For the preparation of solutions of the two varieties, we proceeded according to the following steps:

The dates were washed with tap water, drained and pits/seeds were manually removed and the dates were cut into smaller bits. These were then immersed in distilled water at 80 °C, at a rate of 1 kg/2.5 L of water (Acourene et al., 2008) and brought to a water bath for 45 min, with continuous stirring. Filtration and pressing allowed for the extraction of a maximum amount of juice.

Wet sterilization was performed using an autoclave at 110 °C for 30 min with continuous stirring. This step was to reduce the possibility of competitive microbes existing alongside the *Aspergillus niger*, possibly causing the degradation of the sugars (Siboukeur and El, 2001) and affecting the final product.

Both solutions were diluted to a 14% concentration of total sugars in order to get the best yields (Xu et al., 1989).

The fermentation process was carried out in a shaker. Both solutions of 50 mL substrates were placed in sterilized erlenmeyer flasks of 250 mL capacity, which were then inoculated with a 5% spore suspension. The flasks were incubated at 30 °C for 7 days and under continuous stirring at 150 rpm/min (khattab et al., 2017).

### 2.3. Study of the parameters affecting the fermentation mediums

Four parameters affecting the production of the citric acid were studied to determine the optimal levels for the production of citric acid for our particular fermentation media.

The effect of initial pH was studied by incubating our prepared mediums with the initial pH values ranging from 2 to 6, which were adjusted using 1 N HCl and 1 N NaOH (Kessas et al., 2012).

The effect of different temperatures on the production of the citric acid was studied by incubating the prepared inoculated mediums at different temperatures i.e., 25, 30, 35 and 40 °C.

The optimal incubation period was studied by incubating the mediums for a different length of time, ranging from 1 to 11 days (khattab et al., 2017).

The effect of methanol was studied by adding it at a concentration ranging from 1% to 7%, to the prepared mediums and incubating (Sarkar and Das, 2017).

Each experiment used the necessary number of 250 mL erlenmeyer flasks filled with 50 mL of the substrate solution, which were then autoclaved for sterilization purposes as described previously, before being inoculated with the *Aspergillus niger* and incubated in a shaker incubator for the appropriate duration to match the details of the optimization process.

## 3. Analytical methods

### 3.1. Determination of the moisture content of the dates

The percentage of moisture was determined by the dry weight method by measuring the weight of the date substrate before and

after evaporation drying and was calculated using the following formula:

Moisture content (%) = (Initial weight – final weight) × 100/Initial weight (El-Sohaimy and Hafez, 2010).

### 3.2. Determination of the ash content of the dates

The ash content was determined by method outlined in AOAC and was calculated using the following formula:

Ash (%) = (Initial weight – final weight) × 100/Initial weight (Mallah et al., 2017).

### 3.3. Measurement of Hydrogen Potential (pH) and electrical conductivity values of the medium

Determining the pH of the samples was essential for controlling our fermentation media. The pH values were measured before, during, and after the fermentation process. The recorded variations provide information on the metabolic activity of the fungus, and therefore on the transformation of the sugars into citrate. The pH was determined by direct reading, using a previously calibrated pH meter (Hanna instrument).

The electrical conductivity measurement tells us about the content of soluble salts in the fermentation media. The electrical conductivity was measured using the conductivity meter (Consort C861). The results are expressed in  $\mu\text{S}/\text{cm}$ .

### 3.4. Determination of the total and residual sugar concentration

Both the total and residual sugar contents were measured using the DUBOIS method, which allows the doses to be assessed using both phenol and a concentrated sulfuric acid. In the presence of the two reagents, the sugars gave an orange-yellow color, the intensity of which is proportional to the concentration of the total sugars (Dubois et al., 1956). The optical density was determined using a spectrophotometer set at 490 nm (Hach DRB 200). The sugar level is calculated by reference to a calibration curve previously established with a stock solution of glucose at 200 mg/L.

### 3.5. Determination of the citric acid concentration

The broth culture was filtered to separate the mycelia, and the collected filtrate was used for the estimation of the acid. The citric acid concentration was estimated using the pyridine-acetic anhydride method (Marier and Boulet, 1958). In brief, in an ice bath, 1 mL of the sample was added to 1.3 mL of pyridine and mixed in a vortex. The solution was added with 5.7 mL of acetic anhydride. The test tubes were placed in a water bath at 32 °C for 30 min. The optical density was measured with a spectrophotometer at 420 nm and the citric acid contents of the sample was estimated with reference (ran parallel, replacing 1.0 mL of the culture filtrate with distilled water) to the standard.

### 3.6. Statistical analysis

The results from the study were expressed as mean ± standard deviation of three parallel measurements. The significance of difference was calculated by ANOVA and values  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$  were considered to be significant, highly significant and very highly significant, respectively. The analysis was found with JMP 8.0.2.

## 4. Results

Biochemical characteristics of the two varieties of the dates are grouped in table 1.

The moisture content was 11.59% for the GHARS and 12.82% for the MECH DEGLA. The ash content in the GHARS and the MECH DEGLA samples measured at 1.90% and 2.47%, respectively. The total sugar content in the GHARS was 85% of the total sample and the MECH DEGLA had 80.47%.

### 4.1. Kinetic of Hydrogen Potential (pH) during the fermentation process

The results presented in Fig. 1 show pH changes of the GHARS and MECH DEGLA sample mediums during the fermentation process, and indicate that the pH of the solution consisting of GHARS dates went from  $6.255 \pm 0.14$  on the first day to  $2.5 \pm 0.10$  on the seventh day. The pH of the solution containing the MECH DEGLA dates went from  $5.83 \pm 0.09$  on the first day to  $2.705 \pm 0.28$  on the seventh day.

The pH evolution obtained for each substrate when compared with fermentation time was statistically very highly significant ( $p \leq 0.001$ ).

### 4.2. Kinetic of the of electrical conductivity

The results presented on Fig. 2 shows that the electrical conductivity during the fermentation period of the GHARS samples, was reduced from  $3295 \pm 7.07 \mu\text{S}/\text{cm}$  on the first day to  $1244 \pm 19.79 \mu\text{S}/\text{cm}$  on the seventh day. During the same period, the electrical conductivity of the MECH DEGLA samples was reduced from  $3136 \pm 19.79 \mu\text{S}/\text{cm}$  on day 1 to  $975 \pm 444.06 \mu\text{S}/\text{cm}$  by the seventh day.

The resulting evolution of the electrical conductivity during the fermentation process obtained for each substrate when compared with the fermentation time was statistically very highly significant ( $p \leq 0.001$ ).

### 4.3. Consumption kinetics of the sugar content of GHARS and MECH DEGLA during fermentation

The Fig. 3 shows that the sugar content of the solution of the samples from the GHARS dates was reduced from  $142.85 \pm 1.76 \text{ g}/\text{L}$  on first day to  $39.05 \pm 1.62 \text{ g}/\text{L}$  on the seventh (7) day of fermentation, while that of the solution containing the MECH DEGLA samples was reduced from  $131.05 \pm 1.76 \text{ g}/\text{L}$  on first day to  $33.5 \pm 4.80 \text{ g}/\text{L}$  on the seventh (7) day. The result of the sugar content obtained for each substrate when compared with the fermentation time was statistically very highly significant ( $p \leq 0.001$ ).

### 4.4. Kinetics on the production of citric acid by *Aspergillus niger* of the two mediums during the seven days

The obtained results during this study (Fig. 4) show that the production of the citric acid of the solution containing the GHARS samples increased from 0 g/L on first day to  $34 \pm 1.55 \text{ g}/\text{L}$  on the

**Table 1**  
Characteristics of the two varieties of date.

Parameters	Varieties of date	
	Ghars	Mech Degla
Moisture content (%)	11.59	12.82
Ash rate (%)	1.90	2.47
Total sugar (%)	85	80.47

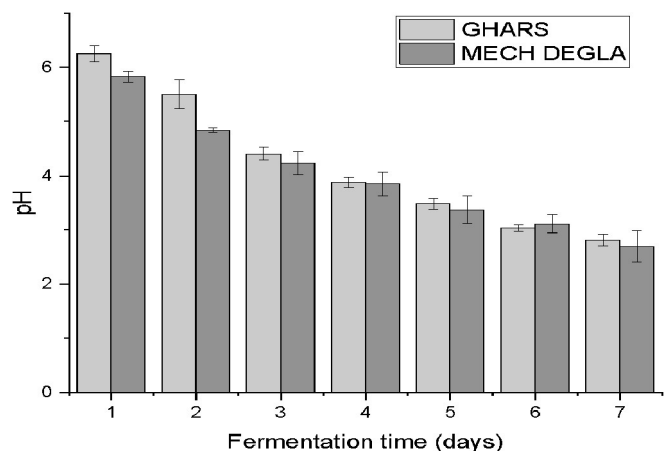


Fig. 1. Kinetic of pH during fermentation by *Aspergillus niger* on medium based on downgraded dates GHARS and the MECH DEGLA during seven days.

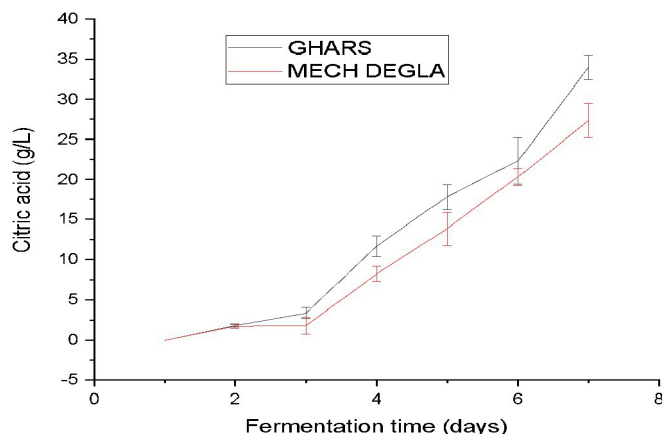


Fig. 4. Kinetic production of citric acid by *Aspergillus niger* on medium based on downgraded dates GHARS and the MECH DEGLA during seven days.

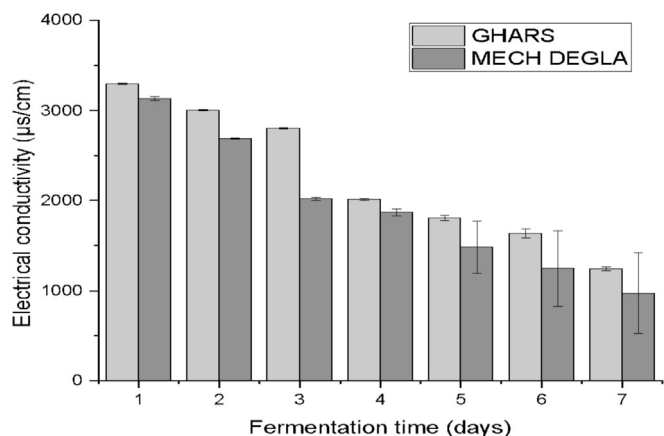


Fig. 2. Kinetic of electrical conductivity during fermentation by *Aspergillus niger* on medium based on downgraded dates GHARS and the MECH DEGLA during seven days.

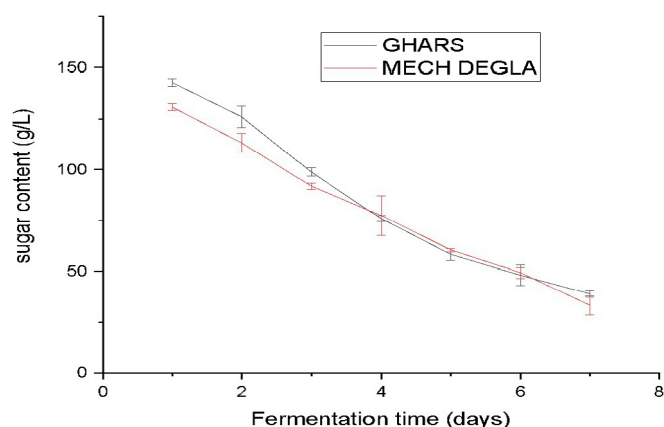


Fig. 3. Kinetics of sugar consumption by *Aspergillus niger* on medium based on downgraded dates: GHARS and the MECH DEGLA during fermentation.

seventh day, while that of the solution of the MECH DEGLA increased from 0 g/L on first day to  $27.4 \pm 2.12$  g/L on the seventh day.

The result of the concentration of the citric acid obtained for each substrate when compared with the fermentation time was statistically significant ( $p \leq 0,001$ ).

4.5. The measured yield of citric acid produced by the *Aspergillus niger* on GHARS and MECH DEGLA mediums

The yield of citric acid of the two varieties of the dates is presented in table 2. It was calculated as: amount of citrate produced/amount of sugar consumed.

5. Optimization of the fermentation medium

5.1. Effect of initial pH on the citric acid production

As shown in Fig. 5, the citric acid yield was recorded the highest for both types of date mediums when the initial pH value was set at 3. The amount of citric acid recorded at this pH value for the GHARS was  $42.25 \pm 0.91$  g/L and for the MECH DEGLA was  $36.6 \pm 1.27$  g/L.

The initial pH in the two media was shown to not have statistical significance on the amount of citric acid produced ( $p > 0.05$ ).

5.2. Effect of temperature on the production of citric acid

Fig. 6 shows that the maximum production of citric acid in both solutions was achieved at 30 °C. The value of the concentration of the citric acid produced in the GHARS containing medium vs the MECH DEGLA was  $34.65 \pm 0.63$  g/L and  $22.45 \pm 0.77$  g/L, respectively at said temperature.

The effect of temperature in the production of citric acid in both media was shown to not have statistical significance ( $p > 0.05$ ).

5.3. The effect of the period of fermentation on the citric acid production

The Fig. 7 shows that the concentration of citric acid increased with the increase in the fermentation period, up to 8 days, and then decreased.

Maximum production of the citric acid for the GHARS and the MECH DEGLA was recorded at  $42.7 \pm 1.8$  g/L and  $37.5 \pm 0.98$  g/L, respectively, which was achieved during 8 days of fermentation, with the temperature set at 30 °C Fig. 7.

Table 2  
Production yield of the citric acid.

Yield g (Citric)/g (Glu)	Varieties of date	
	GHARS	MECH DEGLA
	0,32	0,28

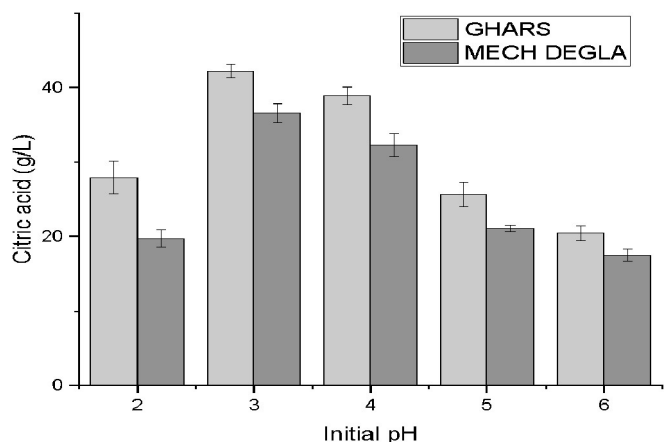


Fig. 5. The effect of initial pH on the citric acid production.

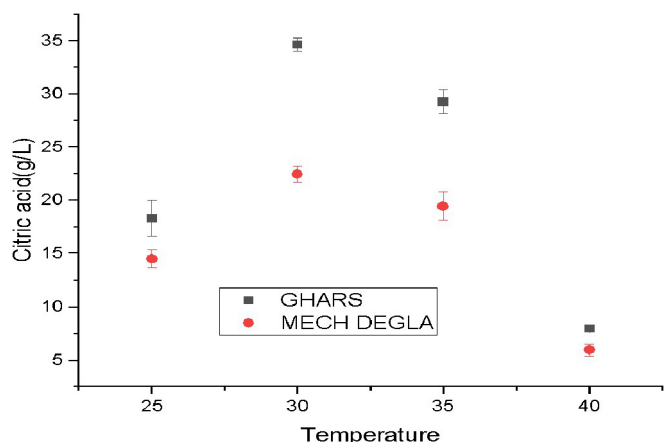


Fig. 6. Effect of temperature on citric acid production.

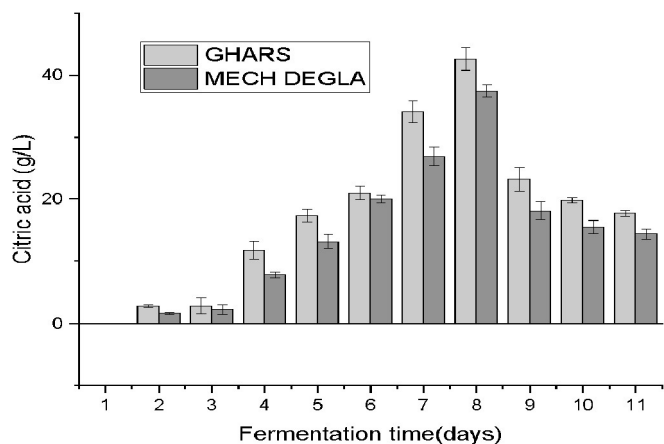


Fig. 7. Kinetic production of citric acid by *Aspergillus niger* on medium based on downgraded dates GHARS and the MECH DEGLA during eleven days.

The length of fermentation in both media was shown to be statistically significant in its effect on the concentration of the citric acid produced ( $p \geq 0.05$ ).

#### 5.4. The effect of methanol on the citric acid production

The 4% methanol was shown to be most effective in increasing the citric acid production after seven days of incubation at 30 °C. The amount of citric acid produced by the two varieties of dates (GHARS and MECH DEGLA) were  $45.5 \pm 0.56$  g/L and  $39.35 \pm 1.20$  g/L, respectively (Fig. 8).

The percent of methanol in the two media was shown to have no statistical significance on the concentration of the citric acid produced ( $p > 0.05$ ).

### 6. Discussion

We used a form of the submerged state fermentation process because it is easily implemented and highly reproducible, even while using dates. Here, the process is shown clearly and the parameters for the fermentation process were additionally optimized for best results in obtaining a high production of the citric acid.

Firstly, the biochemical analysis of GHARS and MECH DEGLA varieties such as moisture, ash and total sugar were observed in the laboratory. The results showed that the percentage of the moisture content was 11.59% for the GHARS and 12.82% for the MECH DEGLA. Dates are naturally low in moisture so this was expected. The ash content was calculated as 1.90% for the GHARS and 2.47% for the MECH DEGLA. The sugar content of GHARS and MECH DEGLA measured at 85 and 80.47% respectively. These amounts were expected and are within range of previous studies (Mallah et al., 2017; Abdul and Assirey, 2015; El-Sohaimy and Hafez, 2010).

The comparison of our results with the study of Gurchala et al. (2015) on five date varieties shows a difference, in fact one of GHARS from the Ghardaïa region in which they found that the moisture content of the GHARS to be at 26.35% and the sugar content to be only 57.21%. Their ash residue measurements were at 1.7%, which was somewhat similar to our finding. These differences might be due to the climate of where their GHARS dates grew. Ghardaïa is located deeper in the Sahara Desert than Tolga, where our dates are from. The altitude and environmental moisture contents in the areas are also different despite both being in the Sahara, potentially leading to differing biochemical properties of the same date type.

The biochemical characteristics results of the two dates varieties as per our records, including the lower moisture content and the higher sugar content of the date, as well as the low ash residual, show that these date varieties make for a good substrate

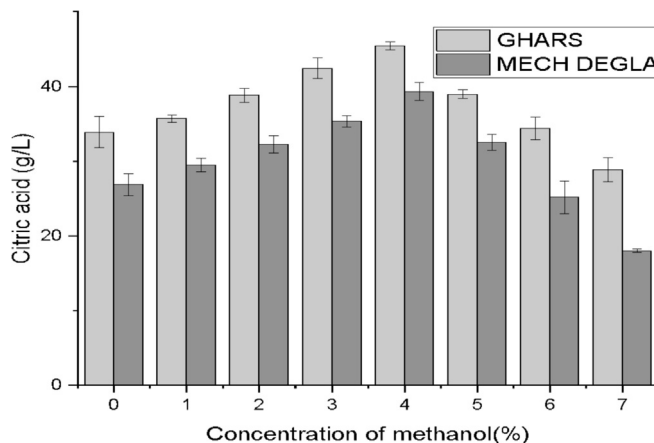


Fig. 8. Effect of methanol on citric acid production.

medium for the *Aspergillus niger* to use for the production of citric acid.

During the fermentation of the basal medium with an incubation period of seven (7) days and at a temperature of 30 °C for the two varieties of dates, we have observed the following: evolution of the pH value in the mediums, the electrical conductivity, and the changes in the sugar content and the citric acid concentration.

There was a progressive decrease in the pH as the fermentation time increased, in both sets of samples. The GHARS and the MECH DEGLA date samples were observed to start at the pH values  $6.255 \pm 0.14$  and  $5.83 \pm 0.09$ , respectively, and gradually decreased to  $2.5 \pm 0.10$  and  $2.705 \pm 0.28$ , respectively. This result is in agreement with the previously published works of (Alam et al., 2010).

The pH measurement is an important aspect in any fermentation process, and it may vary in response to metabolic activities. The most evident reason is the secretion of organic acids that will cause the pH to drop. The pH of the fermentation medium has been found to significantly change at the two ends involving citric acid production. At the beginning, the spores require a pH > 5 in order to germinate. Once germinated and successfully working to produce the citric acid, the medium's pH would naturally become lower, reaching down to pH  $\leq 2$  at the end. The low pH also reduces the risk of contamination of the solution with other microorganisms (Dhillon et al., 2011).

The electrical conductivity during the fermentation period was reduced in both mediums. The reduction in the conductivity measurements provides information on the consumption of the various soluble salts present in the solutions by the *Aspergillus niger* during its development.

Generally, the concentration of sugars in both media decreased gradually during the fermentation period. The sugar content of the solution of the samples from the GHARS dates was reduced from  $142.85 \pm 1.76$  g/L on day 1 to  $39.05 \pm 1.62$  g/L by day seven (7) during fermentation. The solution containing the MECH DEGLA samples was reduced from  $131.05 \pm 1.76$  g/L on day one (1) to  $33.5 \pm 4.80$  g/L by day seven (7). Indeed, it was observed that the fungus consumed almost half of the initial amount of the total sugars during the first four days of the fermentation in both mediums. We observed a decrease in the sugar consumption from the sixth day until the end of the chosen fermentation period. These results are in agreement with (Ike et al., 2019; Kareem et al., 2010).

The decrease of the sugar content is due to the fungi consuming it. However, the levels of the residual sugars of both solutions measured at the end of the fermentation period were  $39.05 \pm 1.62$  g/L for the GHARS and  $33.5 \pm 4.80$  g/L for the MECH DEGLA, showing that the fungi did not finish it all. We can deduce that the period of fermentation chosen (7 days) was not sufficient to use up all the sugar.

About  $34 \pm 1.55$  g/L of the citric acid was produced by GHARS and  $27.4 \pm 2.12$  g/L from MECH DEGLA, after seven days of fermentation. The production of citric acid increased sharply by the second day of the fermentation process in both solutions and a gradual slowing down in the production of the citric acid was noticed, starting from day three. The increase of the citric acid production was accompanied by a decrease of the pH value, the electrical conductivity, and the sugar content amount in the solution. Despite being a high amount, our results were still less than in the concentration of the citric acid production in the study of Mostafa and Alamri (2012). This may be due to their use of date syrup obtained as an accidental by-product in the storage of bagged, humid dates called Alsarifor the enhancement of the production of citric acid, while using the immobilized cells of *Aspergillus niger*. Date syrup is a further refined product of dates and should have a higher sugar concentration, which should also be

more accessible to the fungi, than our unprocessed/unrefined dates, which only went through manual processing.

The measured yield of the production of the citric acid by the GHARS was superior to the yield of the solution containing the MECH DEGLA, at 0.32 g/g and 0.28 g/g respectively. This difference is most likely due to the difference of the respective sugar contents.

A study by Adiba et al. (2015), where they used the MECH DEGLA with an incubation period of fourteen days and a  $1.5 \times 10^7$  spores/mL inoculum was superior to our yield as shown in (Table 2). This difference may be due to our slightly shorter period of fermentation (maximum of 11 days) and the difference in the concentration of inoculums ( $3 \times 10^8$  spores/mL). Comparing the results of our substrates with the previously used and standard substrates.

Yield of citric acid using beet molasses after 4 days of fermentation were ranged from 0.16 g/g to 0.28 g/g from 160 g/L sugar (Guc and Erkmen, 2017) comparing with the yield from our substrates at 0.28 g/g for the MECH DEGLA and 0.32 g/g for the GHARS, with the sugar measuring at 142.85 g/L for the GHARS and 131.05 for the MECH DEGLA.

A study by Ali et al. (2002), using cane molasses a stirred fermenter and using three strains of the *Aspergillus niger* was higher in value at 0.763 g/g compared to our individual yields from either date variety and only one strain of the *Aspergillus niger*. We also used an incubator shaker, which is much less effective in helping fermentation along compared to a fermenter.

In the study of the effect of pH to the media and how it affects the production of citric acid, we observed that citric acid was produced at the highest concentration when the pH value was 3. At this pH value, the citric acid concentration of the GHARS and the MECH DEGLA samples amounted to  $42.25 \pm 0.91$  g/L and  $36.6 \pm 1.27$  g/L, respectively. These results are in agreement with (Ayeni et al., 2019) when they used pineapple waste as a substrate. Similar results have also been previously described by (Haider, 2014).

Some literature reported that maintenance of a low pH during fermentation is vital for a good yield of the citric acid (Papagianni, 2007). A low pH value also inhibits the production of unwanted organic acids (gluconic acid, oxalic acid), and this makes the recovery of citric acid from the broth simple (Karaffa and Kubicek, 2003).

The temperature of the fermentation medium is a very important parameter as it has a profound effect on the production of citric acid and it is notably the most important of all the physical variables (Dhillon et al., 2011). The best concentration of citric acid was achieved when the temperature was set to 30 °C. The concentration of the GHARS and the MECH DEGLA was  $34.65 \pm 0.63$  and  $22.45 \pm 0.77$  g/L, respectively. Several studies found similar results (Alsudani and Al-shibli, 2015; Bekir et al., 2009). In addition, the lowest production of the citric acid was achieved at 40 °C for both samples. The significance of the temperature in the development of a biological process lies in the fact that it could determine some important effects, such as protein denaturation, enzyme inhibition, acceleration and/or suppression on the production of a particular metabolite, and cell death (Pandey et al., 2001).

Maximum production of the citric acid of the GHARS and the MECH DEGLA ( $42.7 \pm 1.83$ ;  $37.5 \pm 0.98$  g/L), respectively, was achieved after eight (8) days of fermentation at 30 °C, which is in agreement with the study of (Maharani et al., 2014).

However, our results are different from some of the studies published previously which found that the optimum fermentation period was seven (7) days (Iqbal et al., 2015; Kanti et al., 2018). A study by Al-Mokhtar and Bakhiet (2015) recorded the maximum citric acid production on the 6th day of fermentation at 30 °C, where as Rao and Reddy (2013) showed that the optimum fermentation period was three (3) days at 30 °C in their study. This differ-

ence might be due to the low concentration of spores inoculated and the amount of sugars available.

It can be seen that the fermentation period had an effect on the production of the citric acid, which was expected. Increased fermentation time means increased contact between the substrate and the fungi.

In the study for the effect of methanol of the fermentation, the best concentration of citric acid production was achieved by the GHARS date sample was  $45.5 \pm 0.56$  g/L, and was  $39.35 \pm 1.20$  g/L for the MECH DEGLA date sample, when the medium had a 4% concentration of methanol. These results are similar to many studies conducted by different authors (Assadi and Nikkhah, 2002), who used date pulp as a substrate; as well as (Sarkar and Das, 2017), who did a study on the production of citric acid with pineapple waste. Hossain et al. (1984) explained that the presence of methanol in the fermentation media increases citric acid production by *Aspergillus niger*. The addition of lower alcohols enhances citric acid production (Vandenberghé et al., 1999).

Several studies have been made on the effect of alcohols on citric acid production. It has been shown to principally act on membrane permeability in microorganisms by affecting the phospholipid composition on the cytoplasmic membrane (Orthofer et al., 1979). Another study (Meixner et al., 1985) argued against its role in membrane permeability by citing citric acid accumulation. The study by Ingram and Buttke (1984) found that alcohols stimulate citric acid production by affecting growth and sporulation through the action of not only affecting the cell membrane permeability, but also by acting on the spatial organization of the membrane or by creating changes in the lipid composition of the cell wall.

## 7. Conclusion

We can conclude that both of the downgraded varieties of dates (MECH DEGLA and GHARS) can be used in a culture medium as substrates for the production of citric acid by the *Aspergillus niger*. Their high yield potential of citric acid is due to their high content of sugars, of which even after seven (7) days of consumption by the fungus, was still not depleted. We can also deduce that, to maximize the citric acid production, it is best to allow the culture to ferment for eight (8) days, at 30 °C, while maintaining a 3 pH once the spores have germinated, which will gradually become lower as more acid is produced. It is also best to use methanol at a 4% concentration to help maximize the citric acid production.

Being of the downgraded variety, the two date varieties have been determined as commercially useless to be sold directly as dates. However, they can be converted into highly valuable byproducts, like citric acid. Since citric acid is an extremely valuable product that is in demand, in both the food and pharmaceutical industry worldwide, the downgraded date varieties can gain another shelf life. In addition, in order to reduce the cost of production of the citric acid by the *Aspergillus niger*, downgraded dates in countries that mass cultivate them, can be used as a reliable source of substrate material for its production.

It is necessary to optimize other parameters of fermentation in the future, such as the concentration of inoculums and nitrogen source for the optima yield of citric acid by the *A. Niger* using downgraded dates.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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