Covenant Journal of Physical & Life Sciences (CJPL) Vol. 6 No. 1, June, 2018

An Open Access Journal available online

Itaconic Acid Production from Date Palm (*Phoenix Dactylifera L*) Using Fungi in Solid State Fermentation

Ajiboye A. E.,[1]* Adedayo M. R.,[1] Babatunde S. K.,[1] Odaibo D. A.,[2] Ajuwon I. B.[1] & Ekanem, H. I.[1]

[1]College of Pure and Applied Sciences,
Department of Biosciences and Biotechnology,
Kwara State University, Malete, P.M.B. 1530, Ilorin,
Kwara State, Nigeria.
[2]University of Ilorin Teaching Hospital,
Ilorin, Kwara State. Nigeria
*adeyinka.ajiboye@kwasu.edu.ng; lizzyboye@hotmail.com

***Abstract*:** This study evaluates the potentials of *Phoenix dactylifera L* (Date fruits) as possible alternative raw materials for itaconic acid production using naturally occurring fungi. Date fruit (pulp) was used as a substrate in solid state fermentation for the production of itaconic acid using naturally occurring fungus.The date fruit (pulp) was de-capped from its seed manually with the aid of a knife and dried in an oven at 60 ^{0}C and was grounded using an Excella Mixer grinder. The fungus used was naturally isolated by fermentation of substrate (date pulp) and was identified as *Aspergillus niger*. Proximate analysis was carried out on the substrate using standard methods. Parameters such as substrate concentration, inoculum size and fermentation period were varied using standard methods to determine its effect on itaconic acid production. Assay for itaconic acid production was carried out using standard methods at a wavelength of 385nm. Amount of itaconic acid produced was derived by translation of absorbance values on the itaconic acid curve. The substrate had a high carbohydrate content of 72.29%. The fermentation results showed maximum production of itaconic acid of 20.75±0.25mg/ml using 40g substrate, 15.13±1.13mg/ml using 2 ml inoculums size of spore suspension (2×10^5 spores/ml) and a maximum yield of 16.88±0.13mg/ml at day 1 of fermentation period. On optimization with 40g substrate and 2 ml

inoculums for 3 days a maximum yield was observed at day 2 of fermentation with a maximum yield of 25.00±1.00mg/ml. The highest acidic level throughout the fermentation period was observed to be at pH 4.2. From the study it was concluded that date pulp is a promising substrate and could be utilized by *Aspergillus niger* for the production of itaconic acid.

Keywords*:* Phoenix dactylifera L *solid state fermentation, itaconic acid,* Aspergillus niger

Introduction

Organic acids have diverse applications in various fields especially in the industries. They include lactic acids, citric acid, gluconic acid and itaconic acid manufactured by large-scale bioprocesses [1]. Among them, the Itaconic acid (methylene succinic acid) is one of the most prominent one. It is a colorless, crystalline carboxylic acid obtained by the fermentation of polysaccharides [2]. Various microorganisms such as fungi of the genus *Aspergillus* have been employed in itaconic acid production through fermentative processes [3]. The most prolific producer being *Aspergillus terreus* which has been frequently utilized for itaconic acid production and subjected to grow under phosphate-limited conditions [4, 5] In the industrial production of itaconic acid, the culture medium is optimized for optimum production and the carbon source mainly used is glucose or sucrose which is readily available [6]. Other species of *Aspergillus* such as *A. niger* has also been employed for itaconic acid production. Itaconic acid is applied primarily in the polymer industry where it is employed as a co monomer for certain products [1]. Its derivatives are used in medicine and cosmetic preparation. The polymerized esters of itaconic acid are used as adhensives, coatings plastics, and elastomers [7]. An N-vinylcaprolactam-containing copolymer of acrylic itaconic acid [8] and poly (acrylic acid-co-itaconic acid) [9] was developed to be used in mechanical and functional GICs. These products are increasingly used in clinical dentistry [10].

The biosynthesis of itaconic acid was not understood for a long time because it was not certain whether itaconic acid emanated from the tricarboxylic acid (TCA) cycle or from a different pathway via citramalate or the transition step via the condensation of acetyl-CoA [11-13]. Bentley and Thiessen [14] suggested a pathway for the biosynthesis of itaconic acid; the breakdown of glucose to pyruvate via glycolysis. This pathway was confirmed by tracer experiments with ^{14}C and ^{13}C labeled substrates [14-16]) and also the necessary enzymatic activities have been proposed [11, 15]. In recent times, itaconic acid was detected in mammalian cells, where it was found in macrophage-derived cells [16]. However, no specific gene encoding this enzymatic activity has been identified and its hysiological role in mammalian cells is still unknown. According to the studies of [16] there are speculations on the role of itaconic acid as an inhibitor of metabolic pathways, because it is described as an enzymatic inhibitor.

Itaconic acid is widely employed to prepare resins used in emulsions coating, leather coating, coatings for

car, refrigerators and other electrical appliances to improve adhesion, color and weather resistance [2]. Thus the need to produce itaconic acid attracts much attention. Various agro-industrial wastes can be fermented for the production of itaconic acid. Date palm (*Phoenix dactylifera* L) a tropical and subtropical tree, belonging to a family Palmae (Arecaceae) is one of the oldest plants cultivated. There is an alarming increase in the worldwide production, utilization and industrialization of dates [17]. Many date fruit producing countries are increasing its production annually. Date fruit have potential for use in production with economical advantages [18]. Date fruits have high nutritional value due to their high sugar content (around50–60%), potassium (2.5 times more than bananas), calcium, magnesium and iron as well as vitamins B_1 & B_2 and Niacin. Furthermore, dates are rich in the monosaccharides: glucose and fructose. Date fruits are considered as very nutritious and they contribute to human health especially when consumed with other food constituents [19, 20]. In Solid-state fermentation (SSF), microorganisms grow on solid materials without the presence of free liquid [21], the process occurs in absence or near absence of free water by employing a natural substrate or inert substrate as carbon source and solid support [22]. Date pulp has potentials for use in industries for acid production with economic advantages. However at the point of transportation, due to mechanical damage during harvesting or mishandling fruit may spoil or may be damaged making it unsuitable for consumption and this damaged fruits instead of being a waste can be made useful in an industrial process. Hence this study is designed towards total utilization of date fruit (date pulp) for the fermentative production of itaconic acid. Scope for value addition using bioprocessing, fermentation with microorganism and increasing yield of itaconic acid by varying various parameters needed to obtain maximum production of itaconic acid.

Materials and Methods

Sample collection and preparation: Date fruit was bought from Zango area in Ilorin, Kwara State. The fruit was identified at the Plant Biology Department, University of Ilorin, Nigeria as *Phoenix dactylifera. L* The pulp was separated from the seed manually with the aid of a knife, pulp was oven dried at 60 ^{0}C and was ground to powder using an Excella mixer grinder and preserved in an airtight container.

Isolation and preservation of test organism: Date fruit (*Phoenix dactylifera L*) pulp was subjected to natural fermentation. Ten grams of grinded fruit pulp substrate was put in a sterile plastic container and mixed thoroughly with 10 mls of distilled water. A clean muslin cloth was then used to cover the plastic for fermentation to take place for 7 days at 28 ^{0}C [23, 24]. After 5 days, 1g of fermented substrate was weighed, serial dilution was carried out and 1 ml of the dilutions was plated out. The isolated fungus was characterized and identified macroscopically and microscopically [25, 26, 27] as *Aspergillus niger*.

Preparation of spore suspension: Wild type *Aspergillus niger* was grown on sabouraud dextrose agar slant at 28 ^{0}C for 7 days. The spore inoculum was prepared by adding 3 ml

of sterile distilled water to each slant containing the cultured fungi and slants were shook for one minute. Number of spores was counted to be 2×10^5 spores/ml [28].

Preparation of fermentation salts: To four conical flasks containing 100 ml of distilled water each, 0.1g of (0.25% (w/v) NH_4cl, 0.095% (w/v) KH_2PO_4, 0.0088% (w/v) $MgSO_4$, and 0.0004% (w/v) $CUSO_4$) was weighed into each conical flask respectively and was stored in an airtight bottle prior to use.

Preparation of bromine reagent: One milliliter of bromine, 3.00g of potassium bromide, 1.87g of potassium chloride, 48.50 ml of 1N hydrochloric acid, and 500 ml of water was used in the preparation of the bromine reagent as used by Fredkin [29]; El Imam *et al.* [30]. Reagent was preserved in an amber reagent bottle.

Solid state fermentation: Varying grams of dried and grinded date pulp was weighed into a 250 ml Erlenmeyer flask, 2 ml of each salts (0.25% (w/v) NH_4cl, 0.095% (w/v) KH_2PO_4, 0.0088% (w/v) $MgSO_4$, and 0.0004% (w/v) $CUSO_4$) were added. Water was added according to varying substrate water holding capacity. Flasks were corked with cotton wool wrapped with foil paper. Flasks were sterilized in an autoclave at 121 ^{0}C for 15 minutes and were allowed to cool. On cooling, substrates were inoculated with spore suspension of *Aspergillus niger* and incubated at 28 ^{0}C. One gram of sample was taken on daily basis to assay for production of itaconic acid using a spectrophotometer.

Assay for itaconic acid: Into a 3 ml curvette, was dispensed 0.3 ml of bromine reagent using a micropipette and was made upto 1.0 ml with distilled water, HCl at pH 1.2 was added to make upto 3.0 ml and left for 15 minutes. After 15 minutes spectrophotometer was blanked at 385nm. Into another 3 ml Beckman cuvette was added 0.3 ml of bromine reagent using a micropipette, 1.0 ml of sample and HCl at pH 1.2 to a volume of 3.0 ml. After 15 minutes the change in optical density was read at 385nm, wavelength of maximum absorption of bromine was also read. Readings were repeated in 20 minutes to ascertain that reaction is completed (Friedkin, 1945).

Proximate analysis of Date pulp (*Phoenix dactylifera. L*): Determination of moisture content, ash content, crude protein content, crude lipid content, crude fibre and total carbohydrate content were determined according to the method of A.O.A.C [31]

Optimization of parameters used during the period of fermentation: The optimum conditions during fermentation were determined by varying parameters such as period of incubation (fermentation days), substrate concentration and inoculum size.

***Effect of varying fermentation days*:** Fermentation days were varied for ten days. Twenty gram of substrate (Date pulp powder) was weighed into ten 250 ml Erlenmeyer flasks and this process was carried out in duplicates. 2 ml of each Salts (0.25% (w/v) NH_4cl, 0.095% (w/v) KH_2PO_4, 0.0088% (w/v) $MgSO_4$, and 0.0004% (w/v) $CUSO_4$) were added and 20 ml of distilled water was added to the content in flasks. Flasks were cotton plugged and autoclaved at 121 ^{0}C for 15 minutes. They were inoculated with 2ml of spore suspension and were incubated at 28 ^{0}C for 10 days. pH readings and assay for itaconic acid were carried out in duplicates on a daily basis.. One

gram of the substrate was dissolved in 100 ml of distilled water and filtered using a Whatman filter paper. Filtrate was used to assay for production of itaconic acid at 24 hours interval. Quantity of itaconic acid was derived by translating from the itaconic acid curve.

***Effect of varying inoculum size*:** Twenty grams of substrate (Date pulp powder) was weighed into 250 ml Erlenmeyer flasks. Twenty milliliters of distilled water was added, cotton plugged and autoclaved at 121 ^{0}C for 15 minutes. After cooling, the flasks were inoculated with 1, 2, 3, 4, 5, and 6 ml of spore suspension (2×10^5 spores/ml) of *A. niger* incubated at 28 ^{0}C for 6 days. They were assayed for itaconic acid on a daily basis as described above

***Effect of varying substrate concentration*:** Different substrate concentrations of the date fruit pulp were also analysed by weighing 10, 15, 20, 25, 30, 35 and 40g of the substrate into 250 ml Erlenmeyer flasks. Fermentation salts were added along side with distilled water according to varying water holding capacity. Flasks were sealed and autoclaved at 121 ^{0}C for 15 minutes and were allowed to cool. After cooling 2 ml of spore suspension (inoculum) was added to each flask and flasks were incubated at 28 ^{0}C for 6 days. Daily pH readings and assay for itaconic acid were carried out as described above. Filtrate was used to assay for production of itaconic acid at 24 hours interval. Quantity of itaconic acid was derived by translating from the itaconic acid curve.

Statistical analysis: The data obtained was analyzed statistically using one way ANOVA. Post-Hoc test using the Duncan Multiple Range test (DMRT) was used to test for the means that are significantly different from each other. A level of significance was determined at $p<0.05$. Statistical package (SPSS 20) was used.

Results

The results as shown in these study shows that Date fruit (pulp)(*Phoenix dactylifera. L*) have the tendency of being used as a substrate in the production of Itaconic acid through solid state fermentation by naturally occurring *Aspergillus niger* isolated from the natural fermentation of Date pulp (*Phoenix dactylifera. L*). Table 1 shows the proximate composition of Date pulp (*Phoenix dactylifera. L*).

Proximate composition of Date pulp

The proximate composition of Date pulp (*Phoenix dactylifera. L*) as shown in Table 1 depicts that carbohydrate have the highest value of 72.29% and Ash content have the lowest value of 2.00%.

Effect of varying fermentation period on itaconic acid production by *Aspergillus niger*

The effect of varying fermentation period of Date pulp for the production of Itaconic acid by naturally occurring *Aspergillus niger* are presented in Table 2. In the fermentation, Date pulp showed the highest yield of 16.88±0.13mg/ml of itaconic acid at day 1followed by day 2 which had a yield of 15.38±0.38mg/ml of itaconic acid and had the lowest yield of 12.75±0.75mg/ml of itaconic acid on day 9.

Effects of varying inoculum size on itaconic acid production by *Aspergillus niger*

The effect of varying inoculum size of Date pulp for the production of Itaconic acid by naturally occurring *Aspergillus niger* are presented in

Table 3. In the fermentation, 2 ml showed the highest yield of 15.13±1.13mg/ml of itaconic acid at day 6 and had the lowest yield of 12.00±0.00mg/ml on day 2 of fermentation.

Effect of varying substrate concentration on itaconic acid production by *Aspergillus niger*

The effect of varying substrate concentration of Date pulp for the production of Itaconic acid by naturally occurring *Aspergillus niger* are presented in Table 4. In the fermentation, 40g of the substrate showed the highest yield of 20.75±0.25mg/ml of itaconic acid at day 1 followed by day 2 which had a yield of 18.88±0.13mg/ml and had the lowest yield of 14.88±0.13mg/ml on day 4.

Optimum production of itaconic acid by *Aspergillus niger* using Date pulp (*Phoenix dactylifera L*)

Optimum production of itaconic acid from all the fermentation parameters by *Aspergillus niger* was carried out with 40g substrate, 2 ml of inoculum size for 3 days at 28 ^{0}C for maximum yield of Itaconic acid and this is shown in Table 5. In the fermentation, day 2 showed the highest yield of 25.00±1.00mg/ml of itaconic acid and there was a decrease in day 1 and 3.

pH values of varying the fermentation period of the fermenting substrate by *Aspergillus niger*

The pH of the fermenting substrate for the fermentation period was taken at an interval of 24 hours. Figure 1 shows the chart representing the fermentation period and as observed the highest acidity was observed to be at pH 4.3.

pH values of varying inoculum size of the fermenting substrate by *Aspergillus niger*

The pH of the fermenting substrate for the inoculum size was taken at an interval of 24 hours. Figure 2 shows the chart representing the inoculum sizes with highest acidic level of pH 4.2

pH values of varying the substrate concentration of the fermenting substrate by *Aspergillus niger*

The pH of the fermenting substrate for the substrate concentration was taken at an interval of 24 hours. Figure 3 shows the chart representing the substrate concentration with highest acidic level of pH 5.4.

Discussion

The results as shown in this study show that Date pulp (*Phoenix dactylifera L*) have the tendency of being used as a substrate in the production of Itaconic acid through solid state fermentation by naturally occurring *Aspergillus niger* isolated from the natural fermentation of Date pulp (*Phoenix dactylifera L*). The proximate composition of Date pulp reveals that carbohydrate content was highest while the Ash content had the lowest value (Table 1). The high carbohydrate content in the pulp serves as a source of sugar to be utilized by the fungi in the fermentation process for optimum production of itaconic acid. This agrees with earlier reports of the importance of polysaccharides in fermentation [32, 33]. However, carbohydrates that are easily metabolized have been found essential for good production of organic acid [34]. Naturally occurring fungi, *Aspergillus niger* growing on fermented Date pulp have the potential of producing itaconic acid which can be used in industries. This is similar to the work of Ajiboye and Sani [35], that *A. niger* was one of the naturally

occurring fungus during fermentation of *Dialium guineense* for citric acid production. However *Aspergillus terreus* still remains the main producer of itaconic acid [1].

Varying of fermentation period showed gradual decrease by every 24 hours. As shown in Table 2, it was observed that day 1 had the highest yield of itaconic acid while day 9 had the lowest yield. This could be due to oxygen and nutrient depletion, more so it could be due to the gradual decrease in the amount of sugar present in the fermenting medium as the fermentation process progresses. The difference in the sugar content and surface area of the starting materials for the fermentation process could account for slight difference in sugar consumption pattern of the systems. This is contrary to the findings of Rafi *et al.* [36] who observed an increase in itaconic acid production with increase in time of incubation showing maximum yield at day 5. When varying inoculum size, the maximum yield of itaconic acid as shown in Table 3, was observed on day 6 of fermentation with 2 ml having the highest yield and 6ml had the lowest yield on the same day. This could be because lower inoculum size result in lower number of cells in the fermentation medium thereby needing a longer time to grow to the level required to utilize the substrate so as to give desired product [37]. However, there was a decrease in 6 ml which was the highest concentration. This could be due to high spore density thereby leading to rapid consumption of available nutrient leaving limited nutrient for utilization for the production of itaconic acid. This is contrary to the work of Chandragiri and Sastry [38] who observed that maximum production of itaconic acid was obtained with 5 ml of inoculum size of *Ustilago maydis.*

In varying substrate concentration there was a high yield of itaconic acid at day 1 of fermentation for all substrate concentrations with 40g having the highest yield of itaconic acid and the lowest yield was observed on day 4 of fermentation except for substrates concentrations 10g and 15g which had their lowest yield on day 6 of fermentation as shown in Table 4. From the result it was observed that the higher the concentration of the substrate the higher the yield. This result is similar to that of Chandragiri and Sastry [38] who obtained maximum production of itaconic acid on 35% concentration of pure glucose and also concurs with the findings of El Imam *et al*. [30] who obtained maximum production of itaconic acid on 40% concentration of Jatropha Curcas seed cake.

On optimization, *Aspergillus niger* was able to utilize the high substrate concentration 40g and 2 ml inoculums size to produce optimum amount of itaconic acid on day 2 of fermentation as shown in Table 5. This agrees with the findings of El Imam *et al*. [30] who obtained maximum production of itaconic acid on 40% concentration of Jatropha Curcas seed cake. The substrates acidic level increased at 24 hours interval with day 3 having its acidic level at pH 4.2 as shown in Figure 5. This is also similar to the findings of El Imam *et al*. [30] who obtained the highest yield of itaconic acid at pH 4. pH is one of the most important parameters that affects the production of itaconic acid by

fermentation as shown in Figure 1-4 it can be deduced that the highest acidity level throughout the fermentation period was at pH 4.2. This is close to the findings of Rao *et al*. [39] and Chandragiri and Sastry [38] who reported the highest production of itaconic acid at pH 3.5 and 3 respectively and it is also similar to the findings of El Imam *et al*. [30] who obtained the highest yield of itaconic acid at pH 4. From the parameters varied as shown in Table 2-4, it was observed that the substrate being a

source of sugar has the ability to produce itaconic acid through fermentation by *Aspergillus niger*. This is in line with the findings of Wilke and Vorlop [5] who observed that itaconic acid is achieved by the fermentation with *Aspergillus niger* on a sugar containing media.

Conclusion

From this study it can be deduced that date fruit, one of the most nutritive fruit is an ideal substrate for the production of organic acids (Itaconic acid) employing solid state fermentation. As observed in the study *Aspergillus niger*, was able to utilize the sugar available in the substrate to produce high quantity of itaconic acid in solid state fermentation. High yield of itaconic acid was shown to depend on the concentration of the substrate, the fermentation period (days) and inoculum size.

Table 1: Proximate Composition of Date Pulp (*Phoenix dactylifera. L*)

Proximate composition	Values (%)
Ash Content	2.00
Moisture Content	6.00
Crude Protein	4.37
Crude Fiber	9.41
Crude Fat	5.93
Soluble Carbohydrate	72.29

Table 2: Effect of Fermentation Period on the production of Itaconic acid from Date pulp (*Phoenix dactylifera. L*) by *Aspergillus niger*

Quantity of Itaconic acid (mg/ml)/ Fermentation Period (days)	
Fermentation Period (days)	Values
1	16.88 ± 0.13^{e}
2	15.38 ± 0.38^{d}
3	15.13 ± 0.13^{cd}
4	14.00 ± 0.50^{b}
5	14.25 ± 0.25^{bc}
6	13.50 ± 0.00^{ab}
7	13.75 ± 0.25^{ab}
8	13.13 ± 0.13^{ab}
9	12.75 ± 0.75^{a}
10	13.50 ± 0.00^{ab}

Values represented in the table are means of two replicate readings and standard error of means of Itaconic acid produced in milligram released from milliliter of the substrate as derived from translation of absorbance values using the Itaconic acid standard curve. Values within the column having different superscripts are significantly different at $p<0.05$

Table 3: Effect of Inoculum size on the production of Itaconic acid from Date pulp (*Phoenix dactylifera. L*) by *Aspergillus niger*

Quantity of Itaconic acid (mg/ml)/ Inoculum size (ml)

Days

Inoculum Size(ml)	1	2	3	4	5	6
1	14.25 ± 0.25^{b}	12.75 ± 0.25^{ab}	12.00 ± 0.50^{a}	13.75 ± 0.25^{a}	12.75 ± 0.75^{a}	14.88 ± 0.38^{a}
2	13.00 ± 0.50^{a}	12.00 ± 0.00^{a}	13.25 ± 0.75^{abc}	13.25 ± 0.25^{a}	13.00 ± 0.50^{a}	15.13 ± 1.13^{a}
3	14.50 ± 0.00^{b}	12.75 ± 0.25^{ab}	14.25 ± 0.25^{c}	13.25 ± 0.75^{a}	13.38 ± 0.63^{a}	14.75 ± 0.25^{a}
4	12.75 ± 0.25^{a}	13.00 ± 0.50^{b}	14.00 ± 0.00^{bc}	13.75 ± 0.75^{a}	13.50 ± 1.50^{a}	15.00 ± 0.00^{a}
5	12.75 ± 0.25^{a}	13.00 ± 0.00^{b}	12.75 ± 0.25^{abc}	12.63 ± 0.13^{a}	12.38 ± 0.38^{a}	13.75 ± 0.25^{a}
6	12.50 ± 0.00^{a}	12.75 ± 0.25^{ab}	12.25 ± 0.75^{ab}	12.88 ± 0.13^{a}	12.00 ± 0.00^{a}	13.25 ± 0.25^{a}

Values represented in the table are means of two replicate readings and standard error of means of Itaconic acid produced in milligram released from milliliter of the substrate as derived from translation of absorbance values using the Itaconic acid standard curve. Values within the same column having different superscripts are significantly different at $p<0.05$

Table 4: Effect of Substrate concentration on the production of Itaconic acid from Date pulp (*Phoenix dactylifera. L*) by*Aspergillus niger*

Quantity of Itaconic acid (mg/ml)/ Substrate Concentration (g)

Substrate Conc.(g)	Days 1	2	3	4	5	6
10	19.63±0.63^{a}	17.63±0.13^{a}	16.75±0.25^{a}	15.25±0.00^{c}	16.13±0.13^{ab}	14.63±0.63^{ab}
15	20.50±0.50^{a}	17.00±0.50^{a}	16.25±0.25^{a}	14.75±0.25^{c}	15.63±0.63^{ab}	13.25±0.25^{a}
20	19.13±1.13^{a}	17.50±0.25^{a}	17.38±0.63^{a}	15.25±0.25^{c}	16.00±1.00^{ab}	17.75±0.25^{bc}
25	19.50±0.50^{a}	17.13±0.13^{a}	16.63±0.13^{a}	13.75±0.25^{ab}	15.50±1.00^{ab}	16.00±1.50^{abc}
30	19.00±0.75^{a}	16.88±0.13^{a}	16.63±0.13^{a}	14.00±0.00^{b}	16.50±0.50^{b}	15.63±0.63^{abc}
35	19.00±1.00^{a}	16.75±0.25^{a}	17.13±0.38^{a}	13.25±0.25^{a}	14.00±0.00 ^{a}	14.50±0.00^{ab}
40	20.75±0.25^{a}	18.88±0.13^{b}	17.38±0.63^{a}	14.88±0.13^{c}	15.00±0.00^{ab}	18.50±2.00^{c}

Values represented in the table are means of two replicate readings and standard error of means of Itaconic acid produced in milligram released from milliliter of the substrate as derived from translation of absorbance values using the Itaconic acid standard curve. Values within the same column having different superscripts are significantly different at $p<0.05$

Table 5: Optimum Production of Itaconic Acid from Date Pulp (*Phoenix dactylifera. L*) by *Aspergillus niger*

Quantity of Itaconic acid (mg/ml)
Optimization (days)

Fermentation Days	Quantity of Itaconic acid (mg/ml)
1	19.50±0.50[a]
2	25.00±1.00[b]
3	19.38±1.13[a]

Parameters for optimization are: Date pulp (*Phoenix dactylifera. L*) :- 40g
Fermentation Period:- 3 days Inoculum Size:- 2 ml Temperature:- 28 ^{0}C

Values represented in the table are means of two replicate readings and standard error of means of Itaconic acid produced in milligram released from milliliter of the substrate as derived from translation of absorbance values using the Itaconic acid standard curve. Values within the same column having superscripts are significantly different at $p<0.05$

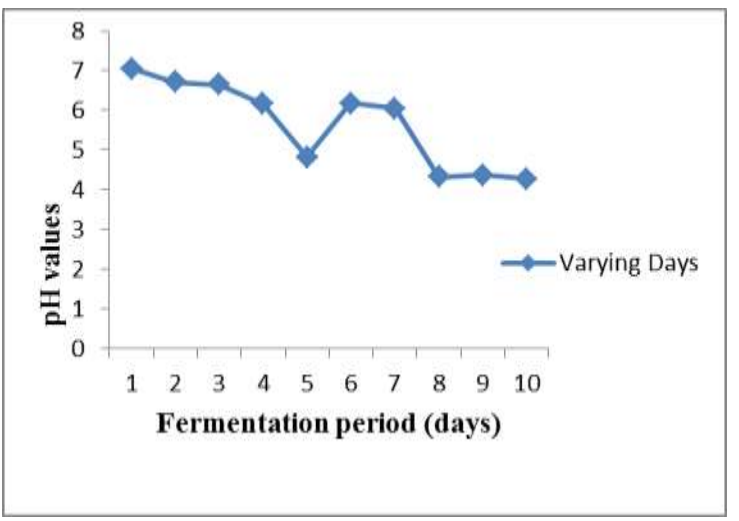


Figure 1: Changes in pH of Date Fruit (Pulp) during different fermentation periods (days) by *Aspergillus niger* for Itaconic acid production

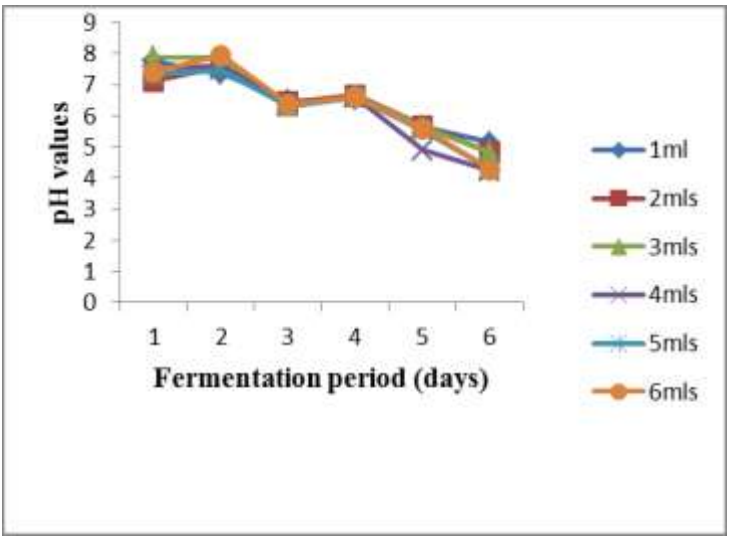


Figure 2: Changes in pH of different inoculums sizes of *Aspergillus niger* during fermentation of Date fruit (pulp) for production of Itaconic acid.

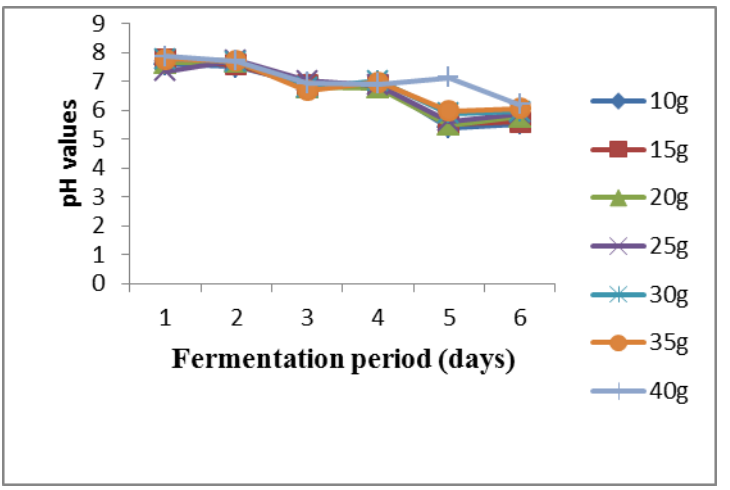


Figure 3: Changes in pH of different concentrations of Date fruit (pulp) during fermentation by *Aspergillus niger* for Itaconic acid production

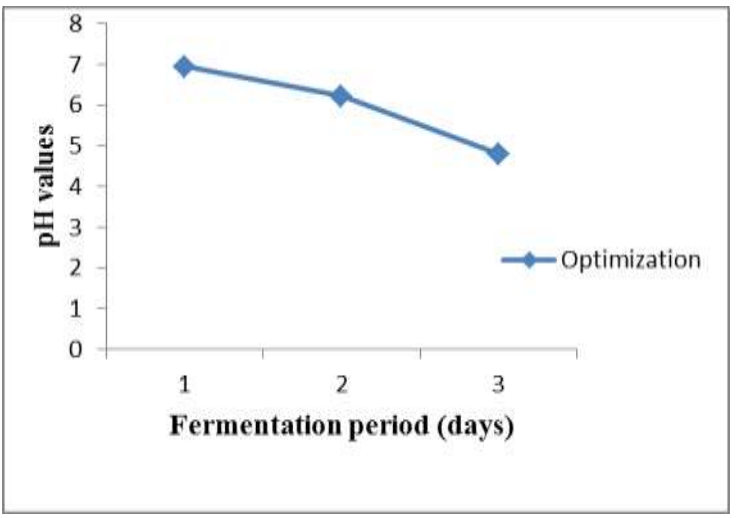


Figure 4: Changes in pH for Optimization of Date fruit (pulp) during fermentation by *Aspergillus niger* for itaconic acid production

References

1 Menon, V. and Rao,M. (2012,) Trends in bioconversion of lignocellulose: Biofuels, platform chemicals and biorefinery concept. Prog. Energ. Combust. 38:522-550.

2 Iqbal, M. and Saeed, A. (2005). *Letters in Applied Microbiology* 40 (3): 178–182.

3 Helia, H. and Wan, M.Y. (2015). Itaconis acid production of microorganism. *Current Journal of Biological Sciences* 7 (2): 37-42.

4 Roehr, M. and Kubicek, C. P. (1996). Further Organic Acids. 2nd Edn., In: Roehr, M. (Ed.), Biotechnology: Products of Primary Metabolism. *VCH VerlagsgesellschaftmbH* 6: 364-379.

5 Willke, T. and Vorlop, K. D. (2001). Biotechnological production of itaconic acid. *Appl. Microbiol. Biotechnol.* 56: 289-295.

6 Menon, V. and Rao, M. (2012). Trends in bioconversion of lignocellulose: Biofuels, platform chemicals and biorefinery concept. *Prog.Energ.Combust.* 38:522-550.

7 Mitsuyasu, O., Dwiarti, L., Shin, K. and Enoch, P. Y. (2009). Biotechnological production of itaconic acid and its biosynthesis in *Aspergillus terreus*. *Appl. Microbiol. Biotechnol.* 84: 597-606.

8 Moshaverinia, A., Roohpour, N., Darr, J. A. and Rehman, I. U. (2009). Synthesis and characterization of a novel N-vinylcarrolactam-containing acrylic acid terpolymer for application in glass-ionomer dental cements. *Acta Biomater*. 5:2101-2108.

9 Culbertson, B.M. (2006). New polymeric materials for use in

glass ionomer cements. *J. Dent*. 4:556-565.

10 Okabe, M., Lies, D., Kanamasa, S. and Park, E. (2009). Biotechnological production of itaconic acid and its biosynthesis in *Aspergillus terreus*. *Appl. Microbiol. Biotechnol*. 84:597-606.

11 Matthias, G., Steiger, M. L., Blumhoff, D.M. and Michael, S. (2013). Biochemistry of microbial itaconic acid production. *Frontier in microbiology*

12 Bentley, R. and Thiessen, C. P. (1957). Biosynthesis of itaconicacidin*Aspergillusterreus*. III. The propertiesand reaction mechanism of cisaconiticacid decarboxylase. *J. Biol.Chem.* 226: 703–720.

13 Winskill, N. (1983). Tricarboxylic acidcycle activity in relation to itaconicacid biosynthesis by *Aspergillus terreus*. *J. Gen. Microbiol*. 129: 2877–2883.

14 Bonnarme, P., Gillet, B., Sepulchre, A. M., Role, C., Beloeil, J. C. and Ducrocq, C. (1995). Itaconate biosynthesis in *Aspergillus terreus*. *J. Bacteriol*. 177: 3573–3578.

15 Jaklitsch, M., Kubicek, P., Scrutton, C., London, C., Road, C. H. and London, W. (1991). The subcellular organization of itaconate biosynthesis *Aspergillus terreus*. *J. Gen. Microbiol*. 137: 533–539.

16 Strelko, C. L., Lu, W., Dufort, F. J., Seyfried, T. N., Chiles, T. C. and Rabinowitz, J. D. (2011). Itaconic acid is a mammalian metabolitei nduced during macrophage activation. *J. Am. Chem. Soc*. 133:16386–16389.

17 FAOSTAT.http://faostat.fao. org/ DesktopDefault.aspx?PageID= 339&lang=en&country=194 accessed on 23.12.2012. 2010

18 Al-Hooti, S., Sidhu, J. S., Al-Otaibi, J., Al-Ameeri, H. and Qabazard, H. (1997). Processing quality of important date cultivars grown in the United Arab Emirates for jam, butter and dates-in-syrup. *Adv.Food Sci*. 19: 35–40.

19 Lambiote, B. (1982). Some aspects of the role of dates in human nutrition. Proceedings of the First International Symposium on Date palm. King Faisal University, Saudi Arabia, March 23–25.

20 Chandrasekaran, M. and Bahkali, A.H. (2013). Valorization of date palm (*Phoenix dactylifera*) fruit processing by-product and waste using bioprocess technology- *Review. Saudi Journal of Biological Sciences* 20: 105-120.

21 Bhargav, S., Panda, B. P., Ali, M. and Javed, S. (2008). Solid-state Fermentation: An Overview. *Chem. Biochem. Eng. Q*. 22 (1):49–70.

2 Kumar, D., Jain, V. K., Shanker, G. and Srivastava, A. (2003). *Process Biochem*. 38: 1731.

23 Lawal, T. E., Iyayi, E. A. and Aderemi, F. A. (2005). Biodegradation of groundnut pod with extracted enzymes from some isolated tropical fungi: growth responses and carcass quality of broilers finisher birds. *Proceedings of*

Annual Conf Animal Sci. Ass. Of Nig. (ASAN). pp. 109-112.

24 Kayode, R. M. O. and Sani, A. (2008). Physicochemical and proximate composition of mango (*Mangifera indica*) kernel cake fermentedwith monoculture of fungal isolates obtained from naturally decomposed mango kernel. *Life Science Journal* 5(4): 55 – 63.

25 Raper, K. B. and Fennell, D. I. (1965). The genus *Aspergillus*. William and Wilkins, Baltimore.

26 Ainsworth, G. C., Sparrow, F. K. and Sussman, A. S. (1973). The fungi – an advanced treatise: A taxonomic review with keys.Ascomycetes and fungi imperfecti, Academic Press, New York, pp. 621.

27 Gilman, J. C. (2001). A manual of soil fungi. Oxford & IBH Publishing Corporation, New Delhi, India.

28 Kuforiji, O. O., Kuboye, A. O. and Odunfa, S. A. (2010). Orange and pineapple wastes as potential substrates for citric acid production. *International Journal of Plant Biology* 1(1): .

29 Fredkin, M. (1945). Determination of itaconic acid in fermentation liquors. *Ind. Eng.Anal.Ed Anal. Ed* 17(10): 637-638.

30 El-Imam, M. A., Kazeem, M. O., Odebisi, M. B., Oke, M. A. and Abidoye, A. O. (2013). Production of itaconic acid from Jatropha curcas seed cake by *Aspergillus terreus*. *Not Sci Biol*. 5(1): 57-61.

31 A.O.A.C. (2000). Official Method of Analysis. 16th Edition. Association of Official Analytical Chemist. Washinton D.C.

32 Xu, D. B., Madrid, C. P., Rohr, M. and Kubicek, C. P. (1989). The influence of type and concentration of the carbon source on production of citric acid by *Aspergillus niger*. *Appl Microbiol Biotechnol*. 30(6):553–558.

33 Amenaghawon, N. A., Areguamen, S. O., Agbroko, N. T., Ogbeide, S. E. and Okieimen, C. O. (2013). Modelling and Statistical Optimisation of Citric Acid Production from Solid State Fermentation of Sugar Cane Bagasse Using *Aspergillus niger*. *International Journal of Sciences Research* Article, 2. http://www.ijSciences.com 2013.

34 Max, B., Salgado, J. M., Rodríguez, N., Cortés, S., Converti, A. and Domínguez, J. M. (2010). Biotechnological production of citric acid. *Braz. J. Microbiol*. 41 (4):

35 Ajiboye, A. E. and Sani, A. (2015). Fermentation of the fruit pulp of *Dialium guineense* for citric acid production using naturally occurring fungi. *International Journal of Current Microbiology and Applied Sciences*. 4(7): 432 – 440.

36 Rafi, M. M., Hanumanthu, M. G., Rao, M. D. and Venkateswarlu, K. (2014). Production of Itaaconic acid by *Ustilago maydis* from agro wastes in solid state fermentation. *J. Biosci. Biotech* 3(2):163-168.

37 Saxena, R. and Singh, R. (2011). Amylase Production by Solid State Fermentation of agro-industrial wastes using *Bacillus* sp. *Braz J Microbial*. 42 (4):1334-1342.

38 Chandragiri, R. and Sastry, R. C. (2011). Synthesis of itaconic acid using *Ustilagomaydis.Canadian J Chem Eng Technol* 2(7):128-135.

39 Rao, D. M., Jaheer, S. M. D., Rangadu, P. V., Subramanyam, K., Sivarama, K. G. and Swamy, A. V. N. (2007). Fermentative production of itaconic acid by *Apergillus terreus* using Jatropha seed cake. *Afr J Biotechnol*. 6(18):2140-2142.