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Utilisation of the Date Wastes as Substrate for the Production of Baker's Yeast and Citric Acid

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Abstract: The aim of the present study was to investigate the potential of date wastes as substrate for the production of Baker's yeast and Citric acid using strains of *Saccharomyces cerevisiae* ATCC 1102 and *Aspergillus niger* ATCC 16404 and the determination of optimized production conditions. Submerged fermentations was carried out in a fermentor of 3 liters capacity. So, the obtained results show that the optimal Baker's yeast production was obtained at dilution rate of 0.22 h⁻¹. On the other hand, the strain of *Saccharomyces cerevisiae* SC-DB-A12 produced high yield biomass compared to other strains. Also, the use of ammonium phosphate provides better biomass yields compared to other nitrogen source. Concerning the citric acid production, the optimal fermentation period was 144 h. The maximum citric acid production was obtained at 30°C and at 150.0 g L⁻¹ of sugars. In the same way, the addition of methanol at concentration up to 3.0% resulted in a marked increase in the citric acid production. In addition, the optimum pH for maximum citric acid production was 3.5. The aeration rate kept at a level of 1.0 L/L/min was found to be optimum. Finally, the best results were observed when 2.5 g L⁻¹ ammonium nitrate and 2.5 g L⁻¹ of potassium phosphate were added into the medium. In summary, a maximum citric acid production i.e., 126.4 g L⁻¹ was obtained at these optimal conditions. It is concluded from these results that the date wastes could serve as a potential substrate for Baker yeast and Citric acid production.

Key words: Submerged fermentation, date wastes, optimization, baker yeast, citric acid

INTRODUCTION

Agricultural and agro-industrial activities produce significant quantities of waste that may be new raw materials for many food industries. To this end, their transformations by biotechnological processes represent a solution of choice as it allows the production of substances with high added value. In Algeria, the production of dates is estimated to 492.188 tons which 45.000 to 50.000 tons is constituted of date wastes (Ministry of Agriculture, 2006). Dates are an economical source of carbohydrates for the production of a lot off metabolites because they are readily available and relatively low priced. In recent years, *Saccharomyces cerevisiae*, considered the most intensively cultivated and commercial micro-organism, has been widely used in leavening dough (Jorgensen *et al.*, 2002). However, the cultivation of this yeast is not without problems. The low productivity obtained under both aerobic Batch fermentation and continuous cultures as a result of

dilution rate dependence has led to the adoption of Fed-batch process for Baker's yeast production (Ejiofor *et al.*, 1996). These observations have been attributed to the Crabtree effect (Jorgensen *et al.*, 2002). On the other hand, the worldwide demand of citric acid is about 6.0×10⁵ tons per year and it is bound to increase day by day (Pazouki *et al.*, 2000). Citric acid is the major organic acid produced by fermentation with *Aspergillus niger* and widely used in the food, beverage, chemical, pharmaceutical and other industries (Mattey, 1992). The basic substrates for citric acid fermentation are beet or cane molasses (Arzumanov *et al.*, 2000). Other carbohydrates and wastes that have been considered, experimentally, to produce citric acid includes, date syrup, carob pod and inulin (Drysdale and McKay, 1995; Roukas, 1998). Many micro-organisms such as fungi and bacteria can produce citric acid. The various fungi which have been found to accumulate citric acid in their culture medium, include strains of *Aspergillus niger*, *Aspergillus awamori*, *Penicillium restrictum*,

Trichoderma viride and *Yarrowia lipolytica* (Arzumanov *et al.*, 2000; Franz *et al.*, 1993). Considerable efforts have been made towards the improvement of fermentation processes as well as the elucidation of microbial fundamentals. Research has also been made on the nutrients and oxygen transfer effects and other environmental factors such as temperature and initial pH related with citric acid production (Papagianni, 2007; Vandenberghe *et al.*, 1999; Walid *et al.*, 2006). Algeria imports Baker's yeast and Citric acid from industrially advanced countries and it involves a huge amount of foreign exchange. The production process for Citric acid had not been developed in Algeria. This will lead to the development of an indigenous technology for the production of this industrially important metabolite. The aim of the present study was to investigate the potential of dates wastes as substrate for the production of Baker's yeast and Citric acid using strains of *Saccharomyces cerevisiae* ATCC 1102 and *Aspergillus niger* ATCC 16404 and the determination of optimized production conditions.

MATERIALS AND METHODS

Material: The vegetable material used is constituted of some date wastes produced by Deglet-Nour variety. The biological strains used are *Saccharomyces cerevisiae* ATCC 1102 for the production of Baker's yeast and *Aspergillus niger* ATCC 16404 for the production of citric acid. The project was conducted at the experimental station of National Institute of Agricultural Research of Touggourt during the period of 2007 to 20010.

Experimental protocol

Date wastes syrup preparation: The syrup is produced by heating date wastes in water at 85°C during 45 min with continuous stirring. The extract is filtered, decanted and clarified.

Production of Baker's yeast on Fed-Batch culture

Inoculums preparation: The strains maintained on agar slope were reactivated in Carlsberg medium composed by (g L^{-1}): Yeast extract 20, Sucrose 100, MgSO_4 1.0, $(\text{NH}_4)_2\text{PO}_4$ 1.0 at pH 4.5. Thus, in 250 mL Erlenmeyer flask, we put 20 mL of this medium and then inoculated. We homogenized and then incubated at 30°C for 24 h under continuous agitation (Al-Obaidi *et al.*, 1987).

Alcoholic fermentation: The objective of this fermentation is to adapt the strain of yeast to the culture medium used. Thus, 300 mL of dates syrup enriched in protein and minerals were inoculated with 20 mL of inoculums. It adjusts to pH 4.5 and incubated at 30°C during 18 h (Mohammed *et al.*, 1986).

Fed-batch culture: The fed-batch culture was conducted over a period of 15 h and we used a fermentor (New Brunswick Scientific) of 3 L capacity. Fermentation temperature is maintained at 30°C and the pH is set at 4.5. The agitation was 300 rpm min^{-1} and aeration of 2.0 L/L/min.

Production of citric acid

Inoculums preparation: A loop full of *Aspergillus niger* ATCC 16404 was spread on sterilized potato dextrose agar plate and incubated at 30°C for 5 days. After growth and sporulation, 40 mL of distilled water was added to each Petri dish. About 10^6 CFU mL^{-1} suspensions were produced (El-Holi and Al-Delaimy, 2003).

Fermentation process: The fermentation was performed in fermentor (New Brunswick Scientific) of 3 L capacity containing 1.0 L dates syrup. The vegetative inoculums was transferred to the production medium at level of 0.5% (w/v) based on the total working volume of the fermentation medium. The incubation temperature was kept between 20-40°C. The initial pH was adjusted between 2.0-6.0. Necessary agitation intensity was also maintained at 200 rpm min^{-1} . Silicone oil was used to control the foaming during fermentation.

Analytical methods: Determination of dry biomass quantity: The dry biomass quantity of Baker's yeast is estimated by centrifugation of 100 mL of culture medium in pre-weighed tubes, then washed twice with equal volume of deionised water. The cell pellets were dried at 0°C to constant weights (Al-Obaidi *et al.*, 1987).

Dosage of mycelial dry weight: Mycelial dry weight was harvested by paper filtration using a pre-dried and pre-weighted Whatman filter paper N°1, washed with distilled water and dried to constant weight at 70°C (Ikram-ul-Haq *et al.*, 2002).

Dosage of citric acid and residual sugars: Citric acid and residual sugars were determined by Shimadzu HPLC instrument (Shimadzu, Kyoto, Japan) equipped with UV detector model SPD-10 Avp, column oven model CTO-10 Avp. The eluent used for analysis was 0.01N sulphuric acid solution. HPLC analyses were carried out under the following operation conditions: Pump flow, 0.6 mL min^{-1} , column temperature 40°C, sample amount 20 μL and integration method (Crolla and Kennedy, 2001).

RESULTS

Optimisation of Baker's yeast production: The fed-batch culture was conducted over a period of 15 h. In this test, we used urea as nitrogen source. Under these experimental conditions, we noted that the biomass yield

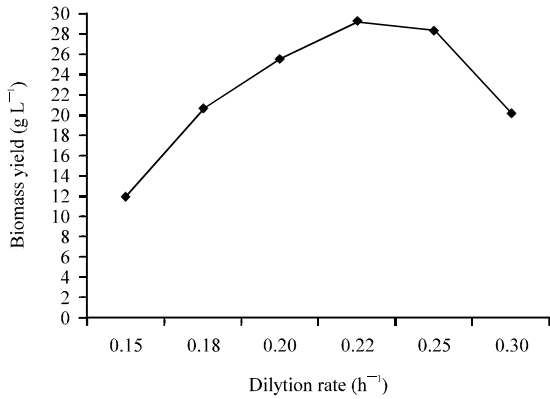


Fig. 1: Evolution of biomass yield following dilution rate

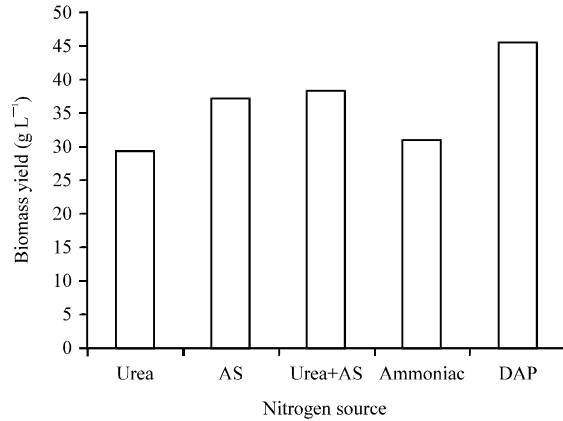


Fig. 3: Evolution of biomass yield following nitrogen source

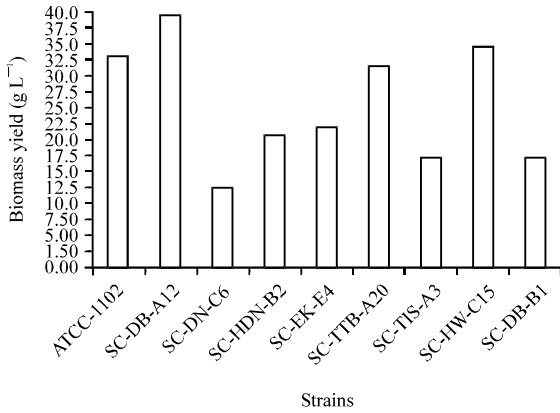


Fig. 2: Evolution of biomass yield following strains

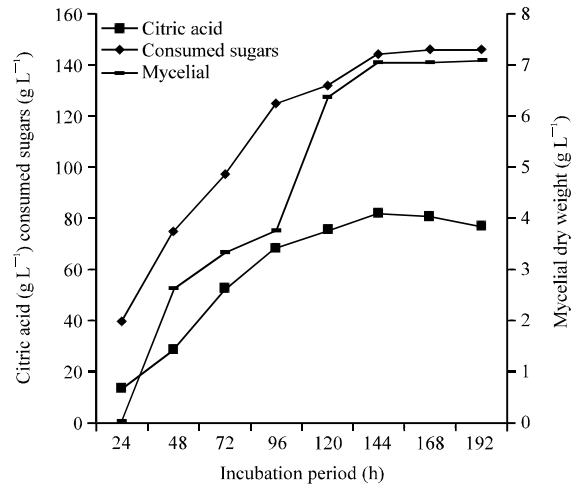


Fig. 4: Evolution of quantity of citric acid, consumed sugars and Mycelial dry weight following incubation period

increased gradually as the dilution rate increases to a maximum of 29.2 g L⁻¹ at 0.22 h⁻¹. In addition, they decrease to 20.1 g L⁻¹ at dilution rate of 0.30 h⁻¹ (Fig. 1). By elsewhere, the results obtained show that the quantity of biomass produced varies considerably with strain used. Thus, strains SC-TTB-A20, SC-HW-C15 and SC-DB-A12 gives high quantities of biomass ranging between 31.5 and 39.5 g L⁻¹, comparable or better than that obtained with strain of ATCC-1102 i.e., 32.9 g L⁻¹. As with the strain of SC-DN-C6, the quantity of biomass produced is very small i.e., 12.3 g L⁻¹ (Fig. 2). Concerning the nitrogen source, the obtained results show that the use of urea provides low biomass yield i.e., 29.2 g L⁻¹ compared to other nitrogen sources. For cons, the highest biomass yield was obtained with ammonium phosphate i.e., 45.37 g L⁻¹ (Fig. 3). Regarding to the vitamin source, as shown in Table 1, the addition of biotin and calcium pantothenate in the medium had no significant effect on biomass yields. Thus, they vary between 40.10 and 42.20 g L⁻¹. However with thiamine, a slight improvement in biomass yield i.e., 44.27 g L⁻¹ was noted at 0.8 mg L⁻¹ thiamine content.

Optimisation of citric acid production: In the present study, cultural conditions such as fermentation period, temperature, sugars concentration, methanol concentration, initial pH, aeration rate, ammonium nitrate and potassium phosphate concentrations were optimised. The fermentation was carried out for 192 h. The obtained results show that the citric acid production increased gradually during the fermentation period and reached to its maximum value, i.e., 82.0 g L⁻¹ after 144 h (Fig. 4). At the optimum fermentation period, the consumed sugar and Mycelial dry weight were 144.0 and 7.03 g L⁻¹, respectively. Increasing in fermentation period did not improve citric acid production and a slight decrease was observed i.e., 76.52 g L⁻¹ at 192 h. By elsewhere, submerged fermentation was carried out on date wastes syrup during 144 h with sugar concentration of

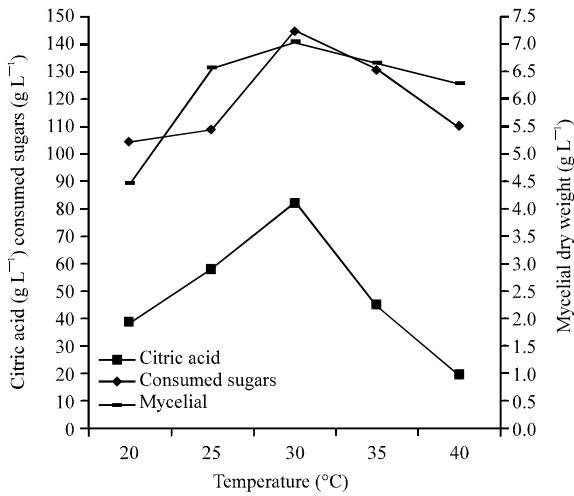


Fig. 5: Evolution of quantity of citric acid, consumed sugars and Mycelial dry weight following temperature

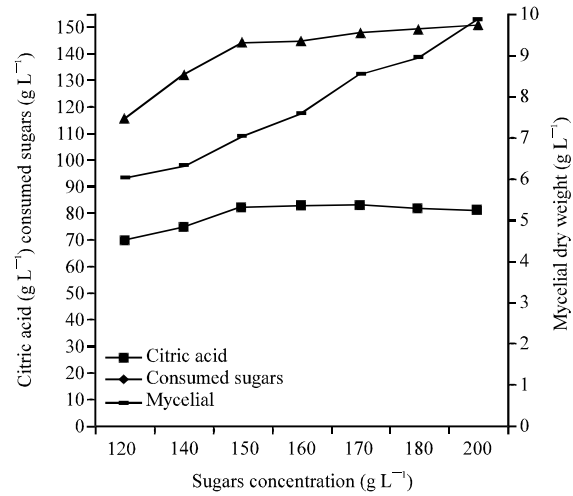


Fig. 6: Evolution of quantity of citric acid, consumed sugars and Mycelial dry weight following sugars concentration

Table 1: Evolution of biomass yield following vitamin source

Quantity of vitamin in mg L ⁻¹	Quantity of biomass in (g L ⁻¹)
Biotin	
0.0	41.3
2.0	40.5
4.0	41.8
6.0	41.4
8.0	42.2
Calcium Pantothenate	
0.0	41.3
1.0	40.1
2.0	40.6
3.0	40.6
4.0	41.0
Thiamin	
0.0	41.3
0.2	40.97
0.4	41.3
0.6	43.52
0.8	44.27

180.0 g L⁻¹, initial pH of 4.0, air supply of 1.0 L/L/min, ammonium nitrate concentration of 2.0 g L⁻¹ and potassium phosphate concentration of 2.0 g L⁻¹ to evaluate the effect of temperature on citric acid production.

As shown in Fig. 5, when the temperature was kept at 20 and 25°C, citric acid production was 38.40 and 57.60 g L⁻¹, respectively. The consumed sugars and Mycelial dry weight were 104.21-108.54 g L⁻¹ and 4.44-6.55 g L⁻¹, respectively. The highest amount of citric acid i.e., 82.00 g L⁻¹ was produced at 30°C. At this temperature, the consumed sugars and Mycelial dry weight were 144.00 g L⁻¹ and 7.03 g L⁻¹, respectively. At higher temperature a gradual decrease in citric acid production was recorded from, 44.8 g L⁻¹ at 35° C to 19.2 g L⁻¹ at 40° C. The consumed sugars and Mycelial dry weight obtained at these temperatures were 130.25-

110.25 g L⁻¹ and 6.62- 6.25 g L⁻¹, respectively. On the other hand, submerged fermentation was carried out on date wastes syrup during 144 h at constant temperature of 30°C, an initial pH of 4.0, air supply of 1.0 L/L/min, ammonium nitrate concentration of 2.0 g L⁻¹ and potassium phosphate concentration of 2.0 g L⁻¹ to evaluate the effect of sugar concentration on citric acid production. As shown in Fig. 6, maximum citric acid production i.e., 82.0 g L⁻¹ was obtained at 150.0 g L⁻¹ of sugars. Also, the consumed sugars and Mycelial dry weight were 144.0 and 7.03 g L⁻¹, respectively. A reduction in citric acid production i.e., 80.8 g L⁻¹ was observed when the sugar concentration of date wastes medium was increased at 200.0 g L⁻¹. Besides, a higher sugars concentration superior or equal to 200.0 g L⁻¹, leads to greater amount of residual sugars i.e., 49.58 g L⁻¹, making the process uneconomical. Furthermore, the submerged fermentation was carried out on date wastes syrup during 144 h at constant temperature of 30°C, sugar concentration of 150.0 g L⁻¹, an initial pH of 4.0, air supply of 1.0 L/L/min, ammonium nitrate concentration of 2.0 g L⁻¹ and potassium phosphate concentration of 2.0 g L⁻¹ to evaluate the effect of Methanol concentration on citric acid production. The Methanol was added to the culture medium 24 h after inoculation at a rate of 1.0, 2.0, 3.0 and 4%. As shown in Fig. 7, citric acid production increased with increases Methanol concentration. So, the addition of 3% methanol in the fermentation medium increases the citric acid production from 82.0 to 96.0 g L⁻¹. Beyond 3% of Methanol, no improvement on citric acid production was noted i.e., 82.0-83.7 g L⁻¹. The consumed sugar was 142.1-143.2 g L⁻¹ and Mycelial dry weight was observed to be 7.05-7.45 g L⁻¹. On the other hand, the submerged fermentation was carried out on date wastes

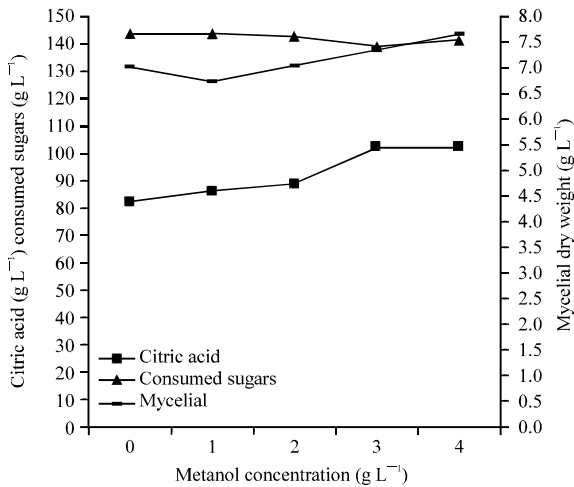


Fig. 7: Evolution of quantity of citric acid, consumed sugars and Mycelial dry weight following methanol concentration

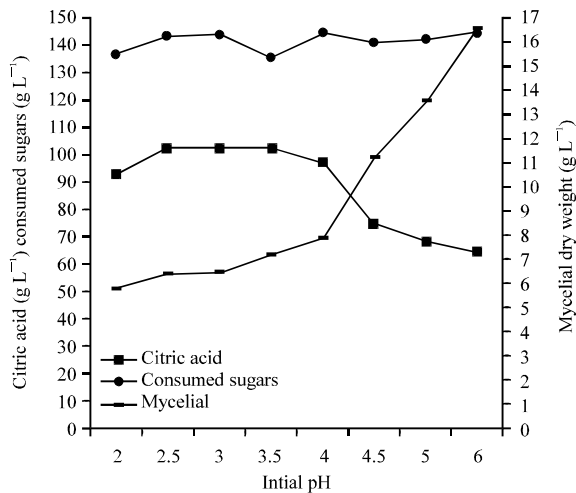


Fig. 8: Evolution of quantity of citric acid, consumed sugars and Mycelial dry weight following initial pH

syrup during 144 h at constant temperature of 30°C, sugar concentration of 150.0 g L⁻¹, Methanol concentration of 3.0%, air supply of 1.0 L/L/min, ammonium nitrate concentration of 2.0 g L⁻¹ and potassium phosphate concentration of 2.0 g L⁻¹ to evaluate the effect of an initial pH on citric acid production. The obtained result shows that the maximum amount of citric acid i.e., 102.4 g L⁻¹ was achieved when the initial pH of fermentation medium was kept at 2.5-3.5 (Fig. 8). The consumed sugar was 135.1-143.6 g L⁻¹ while Mycelial dry weight was 6.36-7.19 g L⁻¹. In addition, low pH inhibits lightly the growth of *Aspergillus niger*. So, the Mycelial dry weight obtained at pH 2.0-2.5 varies between 5.76 and 6.36 g L⁻¹. When the pH was further increased from 4.5,

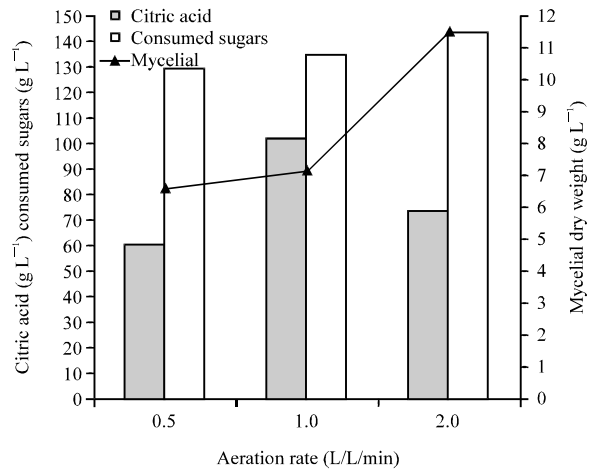


Fig. 9: Evolution of quantity of citric acid, consumed sugars and Mycelial dry weight following aeration rate

the production of citric acid decreased gradually and the amounts of citric acid obtained at pH 4.5, 5.0 and 6.0 were 74.6, 68.2 and 64.4 g L⁻¹, respectively. The consumed sugar and Mycelial dry weight were 140.7-143.8 g L⁻¹ and 11.1-16.5 g L⁻¹, respectively. Submerged fermentation was carried out on date wastes syrup during 144 h at constant temperature of 30°C, sugar concentration of 150.0 g L⁻¹, Methanol concentration of 3.0%, an initial pH of 3.5, ammonium nitrate concentration of 2.0 g L⁻¹ and potassium phosphate concentration of 2.0 g L⁻¹ to evaluate the effect of aeration rate on citric acid production. The obtained results show that the amount of citric acid produced is 60.8 g L⁻¹ when the aeration rate was maintained at 0.5 L/L/min. The consumed sugars and Mycelial dry weight were 129.3 and 6.62 g L⁻¹, respectively (Fig. 9). The maximum of citric acid yield i.e., 102.4 g L⁻¹ was produced when the aeration rate was kept at 1.0 L/L/min. The consumed sugars and Mycelial dry weight were 135.1 and 7.19 g L⁻¹, respectively. At high aeration rate of 2.0 L/L/min, the amount of citric acid produced is 73.6 g L⁻¹. The consumed sugars and Mycelial dry weight were 144.3 and 11.56 g L⁻¹, respectively. By elsewhere, the effect of nature of nitrogen source and ammonium nitrate concentration on citric acid production, by using *Aspergillus niger* for a 144 h fermentation period, was studied under optimum conditions (temperature of 30°C, sugar concentration of 150.0 g L⁻¹, Methanol concentration of 3.0%, an initial pH of 3.5, air supply of 1.0 L/L/min and potassium phosphate concentration of 2.0 g L⁻¹). At this effect, the use of ammonium nitrate as nitrogen source gives a high citric acid quantity i.e., 102.40 g L⁻¹ compared

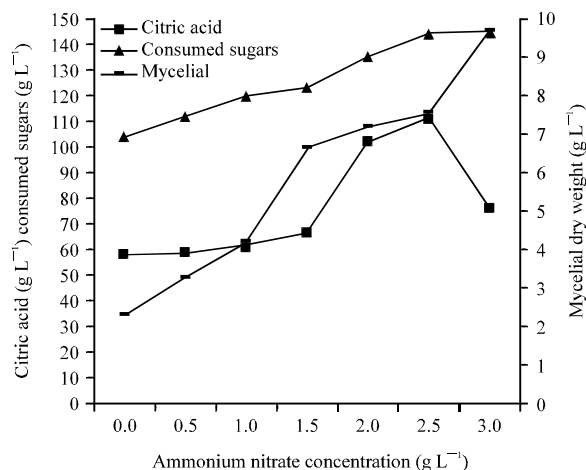


Fig. 10: Evolution of quantity of citric acid, consumed sugars and Mycelial dry weight following the ammonium nitrate concentration

Table 2: Evolution of citric yield following nitrogen source

Nitrogen source	Ammonium carbonate	Ammonium nitrate
Biomass (g L ⁻¹)	13.50	7.19
Consumed sugars	141.70	135.10
Quantity of citric acid (g L ⁻¹)	67.20	102.40

to those obtained with ammonium carbonate i.e., 67.20 g L⁻¹ (Table 2). The consumed sugars and Mycelial dry weight obtained with ammonium nitrate were 135.1 and 7.19 g L⁻¹, respectively. On the other hand, the obtained results indicate that the weak concentrations (≤ 1.5 g L⁻¹) in ammonium nitrate gives some quantities in citric acid and Mycelial dry weight, weak i.e., 58.24-66.35 and 2.28-6.62 g L⁻¹, respectively (Fig. 10). The maximum of citric acid production i.e., 112.0 g L⁻¹ was obtained when the ammonium nitrate concentration was kept at 2.5 g L⁻¹. The consumed sugar was 144.0 g L⁻¹ while Mycelial dry weight was 7.55 g L⁻¹. Also, in this study we noted a diminution in citric acid production i.e., 76.0 g L⁻¹ at nitrogen concentration superior or equal to 3. g L⁻¹. The consumed sugars and Mycelial dry weight were 145.12 and 9.68 g L⁻¹, respectively. Finally, submerged fermentation was carried out on date wastes syrup during 144 h at constant temperature of 30°C, sugar concentration of 150.0 g L⁻¹, Methanol concentration of 3.0%, an initial pH of 3.5, air supply of 1.0 L/L/min and ammonium nitrate concentration of 2.5 g L⁻¹ to evaluate the effect of potassium phosphate concentration on citric acid production. The obtained results show that adding small amounts of potassium phosphate (≤ 1.0 g L⁻¹), gives low quantities of Mycelial dry weight and citric acid i.e., 2.29-3.55 g L⁻¹ and 58.24-68.22 g L⁻¹, respectively (Fig. 11). On the other hand, high Mycelial dry weight i.e.,

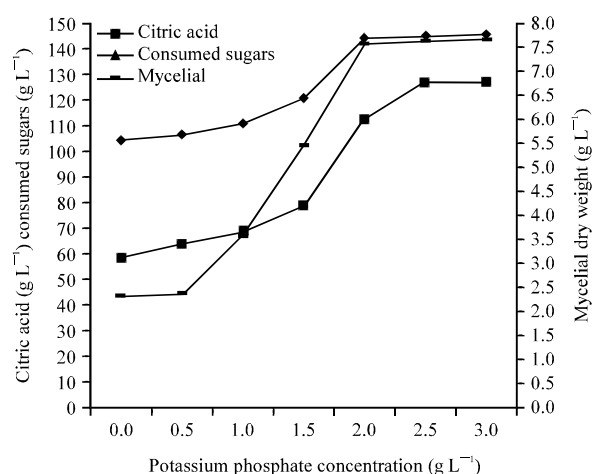


Fig. 11: Evolution of quantity of citric acid, consumed sugars and Mycelial dry weight following potassium phosphate concentration

7.62 g L⁻¹, maximum consumed sugars i.e., 144.8 g L⁻¹ and maximum citric acid production i.e., 126.4 g L⁻¹ were obtained when the potassium phosphate concentration was kept at 2.5 g L⁻¹. By cons, no significant improvement in citric acid production i.e., 126.8 g L⁻¹ at potassium phosphate concentration superior or equal to 3.0 g L⁻¹. The consumed sugars and Mycelial dry weight were 145.10 and 7.64 g L⁻¹, respectively at this concentration.

DISCUSSION

Optimisation of Baker's yeast production: The best results were obtained with a dilution rate of 0.22 h⁻¹. This dilution rate is similar to those applied by Daramola and Zampraka (2008), Ejiofor *et al.* (1996) and Reed and Pepler (1973). However, Akinyemi *et al.* (2003) and Miskiewicz and Borowiak (2005) obtained higher biomass yields with lower dilution rate i.e., 0.15 h⁻¹. By cons, Gueguim-Kana *et al.* (2007) and Shashi and Joshi (2006) obtained better yields at dilution rates ranging between 0.24 and 0.28 h⁻¹. According to Van Hoek *et al.* (2000), at dilution rate below than 0.28 h⁻¹, the glucose metabolism was fully respiratory and absence of ethanol production was observed with strain of *Saccharomyces cerevisiae* DS28911. In this study, a net decrease in biomass yield was observed at a dilution rate of 0.30 h⁻¹. This is probably due to an excess of sugar in the medium preventing good oxygen diffusion. The same result has been reported by Miskiewicz and Borowiak (2005) and Reed and Pepler (1973). By elsewhere, biomass yields obtained with some strains of *Saccharomyces cerevisiae* isolated from dates are high. Similar results were reported

by Al-Obaidi *et al.* (1987) and De Kock *et al.* (2000) with some strains of *Saccharomyces cerevisiae*. However, Ejiofor *et al.* (1994) obtained a better biomass yield by using a strain of *Saccharomyces cerevisiae* DSM 2155. Concerning the nitrogen source, the obtained results show that the use of urea provides low biomass yield compared to other nitrogen sources. For cons, the highest biomass yield was obtained with ammonium phosphate. Similar results were reported by Miskiewicz and Borowiak (2005). The improvement of biomass yield obtained with this nitrogen source is probably related to the contribution of this nitrogen source in phosphorus in appreciable quantities in the medium essential for the growth of yeasts Reed and Pepler (1973). Regarding to the vitamin source, the obtained results shows that the addition of biotin and calcium pantothenate in the medium had no significant effect on biomass yields. This is probably related to the richness of the dates in these vitamins. Similar results were obtained by Al-Obaidi *et al.* (1987) on dates medium. Finally with thiamine, a slight improvement in biomass yield was noted and the required optimum content is 0.8 mg L^{-1} .

Optimisation of citric acid production: The optimal incubation period for maximum citric acid production was 144 h. Present finding is an agreement with those obtained by Ali *et al.* (2003); Arzumanov *et al.* (2000) and El-Holi and Al-Delaimy (2003). Further increase in fermentation period at 192 h a slight decrease in citric acid production was observed. It might be due to the decreased available nitrogen in fermentation medium, the age of fungi and depletion of sugar contents (Arzumanov *et al.*, 2000). The similar results have also been reported by Ikram-ul-Haq *et al.* (2002).

By elsewhere, Mazhar *et al.* (2003) and Papagianni *et al.* (2005) obtained maximum amounts of citric acid with molasses based medium, 168 h after inoculation. So, our findings are more significant as compared to previous workers because reduction of fermentation period reduced the cost of citric acid production. By elsewhere, the optimum temperature was 30°C and the weakest was 40°C . These results were in an agreement with those obtained by Ali *et al.* (2002) and Arzumanov *et al.* (2000) and Asad-ur-Rehman *et al.* (2002). According to Asad-ur-Rehman *et al.* (2002), the temperature of 40°C was most favourable for oxalic acid production while citric acid accumulation is completely inhibits at this temperature. By elsewhere, the optimum sugars concentration for maximum citric acid production was 150.0 g L^{-1} . This result was in agreement with those obtained by El-Holi and Al-Delaimy (2003) and Ali *et al.* (2002). These authors stated that the advantages of high fermentable sugars in the fermentation medium

may be due to the suppression of osmosensitive of contaminates and reduced the dilution water requirements. They also, observed that the sugar concentration in the fermentation medium had a marked effect on the rate of citric acid and morphology of the producer micro-organism. However, Demirel *et al.* (2005) and Papagianni *et al.* (2005) reported that maximum citric acid production was obtained at 140.0 g L^{-1} of sugars concentration. By elsewhere, a reduction in citric acid production was observed when the sugar concentration of date wastes medium was increased at 200.0 g L^{-1} . It may be due to the over growth of the mycelium which resulted in increased viscosity of the medium and polyalcohol formation Demirel *et al.* (2005). The similar results were reported by Ikram-ul-Haq *et al.* (2002) and Ali *et al.* (2002). Also, Papagianni (2007) reported that the higher sugars levels in the fermentation medium increased Mycelial formation and reduced the citric acid yield. Besides, a higher sugars concentration superior or equal to 200.0 g L^{-1} , leads to greater amount of residual sugars making the process uneconomical. Pazouki *et al.* (2000) pointed out that sugars concentration higher than 160.0 g L^{-1} leads to greater amount of residual sugars, making the process uneconomical. On the other hand, citric acid production decreased at lower sugars concentration (120.0 g L^{-1}). This is probably due to the accumulation of oxalic acid in the culture medium Demirel *et al.* (2005); Ikram-ul-Haq *et al.* (2002) and Papagianni (2007). On the other hand, the best results were obtained at 3.0% methanol content. However, according to Ikram-ul-Haq *et al.* (2003), the addition of 1.0% methanol was found to give maximum amount of citric acid. Previous reports stated that the presence of methanol in the fermentation medium may increase citric acid production (El-Holi and Al-Delaimy, 2003; Ikram-ul-Haq *et al.*, 2003). On the other hand, Demirel *et al.* (2005) reported that the addition of 4% of methanol to the fermentation medium induced citric acid production which probably chelates high levels of inhibitory metal ions like Cu, Fe and Zn present in the medium. Methanol is not assimilated by *Aspergillus niger* and its exact role in stimulation of citric acid production is still not clear (Papagianni, 2007). Other studies showed that the methanol stimulate citric acid production by affecting growth and sporulation on space organization of the membrane or changes in lipid composition of the cell wall Ikram-ul-Haq *et al.* (2003). The production of citric acid by *Aspergillus niger* is very sensitive to initial pH of fermentation medium. The maintenance of favourable pH is very essential for the successful fermentation of citric acid (Mattey, 1992). The maximum of citric acid was achieved when the initial pH of fermentation medium was kept at 2.5-3.5. These results were consistent with

findings of Papagianni *et al.* (2005) they found that the maximum of citric acid yield was obtained at pH 3.5. A low pH also inhibits the production of unwanted organic acids (gluconic acid, oxalic acid) and this makes the recovery of citric acid from the broth simpler Papagianni *et al.* (2005). In addition, low pH inhibits lightly the growth of *Aspergillus niger* and reduces the risk of contamination of the fermentation with other micro-organisms. Increase in initial pH caused reduction in citric acid production. The higher initial pH leads to the accumulation of oxalic acid as reported by Ali *et al.* (2002) and Papagianni (2007). According to Papagianni *et al.* (2005), increasing initial pH to 5.0 during the production phase reduces the final yield of citric acid by up to 80%. However, Ikram-ul-Haq *et al.* (2002) have obtained a maximum amount of citric acid, with an initial pH of the culture medium of 6.0. Similarly, Ikram-ul-Haq *et al.* (2002) have obtained a maximum citric acid yield at pH 5.5. Finally, Walid *et al.* (2006) recommends an initial pH of fermentation medium to 4.0 in order to optimize the production of citric acid. The degree of aeration depends upon the organism, the medium composition and the size of fermentor. The maximum of citric acid yield was produced when the aeration rate was kept at 1.0 L/L/min. Similar result has also been reported by Ikram-ul-Haq *et al.* (2002) and Sanjay and Sharma (1994). Normally the oxygen demand of a fermenting culture is so high that the amount of oxygen in a saturated aqueous medium is inadequate Sanjay and Sharma (1994). Papagianni (2007) concluded that interruption of aeration during submerged fermentation, affects citric acid production; however, the extent of damage depends upon the duration of interruption and the phase of fermentation. On the other hand, nitrogen has a profound effect on citric acid production because nitrogen is not only important for metabolic rates in the cells but it is also the basic part of cell proteins. Also, the type and concentration of nitrogen source affect fungal growth and the synthesis of citric acid (Mattey, 1992). Thus, a comparative study shows the use of ammonium nitrate as nitrogen source is better than the ammonium carbonate. By elsewhere, the weakest citric acid production was obtained at concentrations of ammonium nitrate inferior or equal to 1.5 g L⁻¹. It might be due to the lower supply of available nitrogen for mycelia growth. The optimal ammonium nitrate concentration was 2.5 g L⁻¹. This result is in agreement with those obtained by Ali *et al.* (2002). Any increase or decrease other than a concentration of 2.5 g L⁻¹, resulted in the disturbance of fungal growth and subsequently in citric acid production. An advantage of using ammonium salts is that the pH declines as the salts are consumed and a low pH is requirement for citric acid fermentation (Mattey, 1992).

Finally, Vandenberghe *et al.* (1999) noted that the high nitrogen content increase fungal growth and sugar consumption but decrease the citric acid yield. Studies on the effects of phosphorus limitation have been contradictory. However, when the quantities of trace elements are not limiting, the addition of phosphorus has resulted in the growth of *Aspergillus niger* (Papagianni, 2007). The obtained result shows that adding small amounts of potassium phosphate (≤ 1.0 g L⁻¹), gives low quantities of citric acid. It might be due to the lower supply of available phosphorus for mycelia growth. The optimum potassium phosphate concentration for maximum citric acid production was 2.5 g L⁻¹. Similar results were obtained by Ali *et al.* (2002) and Demirel *et al.* (2005). However, Asad-ur-Rehman *et al.* (2003) noted that a high potassium phosphate concentration (≥ 2.5 g L⁻¹) promotes the growth of *Aspergillus niger* at the expense of citric acid accumulation.

CONCLUSION

The obtained results showed that date wastes syrup serve as a good substrate, enabling the growth of *Saccharomyces cerevisia* and *Aspergillus niger* which produced a considerable amounts of Baker's yeast and Citric acid. Evidently date wastes syrup provided necessary nutrients for these micro-organisms to grow and synthesize these metabolites. In summary we can say that the optimum yield of Baker's yeast was obtained with a rate dilution of 0.22 h⁻¹ and the use of ammonium phosphate as nitrogen source. Concerning the citric acid production, the cumulative effect of temperature(30°C), sugars concentration of 150.0 g L⁻¹, methanol concentration of 3%, initial pH of 3.5, aeration rate of 1.0/L/L/min, ammonium nitrate concentration of 2.5 g L⁻¹ and potassium phosphate concentration of 2.5 g L⁻¹ during the fermentation process of date wastes syrup did increase the citric acid production to 126.4 g L⁻¹.

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