Iran J Biotech. 2014 November;12(3): e1006 DOI:10.15171/ijb.1006

Published online 2014 November 25 **Research Article**

Date Wastes as Substrate for the Production of α-Amylase and Invertase

Acourene Said [1*], Amourache Leila [2], Djafri kaouther [1], Bekal Sadia [3]

[1]National Institute of Agricultural Research, Touggourt BP 17, Ouargla 30200 ALGERIA
[2]Institute of Nutrition, Food and Agro-Alimentary, Technologies University of Constantine, ALGERIA
[3]Southern Illinois University Carbondale, U.S.A.

[*]*Corresponding Author*: Acourene Said, National Institute of Agricultural Research, Touggourt BP 17, Ouargla 30200 ALGERIA, P.O. Box: 30200. Tel: +213-777800345, Fax: +213-29693288, E-mail: acourmam@yahoo.fr

Received: June 04, 2013; **Revised:** May 28, 2014

Background: In Algeria the date wastes production is estimated at least 85.000 tones. Date wastes is an economic source of carbohydrates for conversion to industrial enzymes because it is readily available and relatively low priced.
Objective: The aim of the present study was to investigate the potential of using date wastes as a substrate for the production of α-amylase and invertase.
Materials and Methods: Thirty strains of *A. niger* were isolated from the saline soils collected from five arid locations in Algeria. The process parameters; time, temperature, sugar content, initial pH, nitrogen source, nitrogen and phosphorus content, in the production of these enzymes were optimized.
Results: The obtained results showed the potential of the Aspergillus niger for level productions of these enzymes. For α-amylase production, the cumulative effect of fermentation period of 96 h, temperature of 30°C, sugar content of 20 g/L, initial pH 5.5, supplemented with yeast extract as nitrogen source, and yeast extract and potassium phosphate at 5.0 g/L, content during the fermentation process of date wastes syrup produced α-amylase levels up to 285.6 U/ml. Invertase was produced up to 195.56 U/ml were produced under optimum conditions of 96 h, temperature of 30°C, initial pH 6.0, sugars content of 40.0 g/L and the utilization of yeast extract and potassium phosphate at concentrations of 11.0 and 3.5 g/L, respectively.
Conclusions: The results obtained, provided evidences, supporting the potential of date waste as suitable source for production of α-amylase and invertase at industrial scale.

Keywords: Amylase; Date wastes; Invertase; Optimization; Submerged fermentation

1. Background

In Algeria, the production of dates is estimated to be 520.00 tons of which 85.000 to 105.000 tons are of low commercial value (1). Date wastes is considered as an economical source of carbohydrates for conversion to industrial enzymes because it is readily available and relatively low priced.

Several extra cellular enzymes are commercially available and widely used in the industry. Among them, α-amylases and invertases are used in many industrial processes. For example, α-amylases are involved in the assimilation of starch-based matter provided as substrates. Amylases find potential application in a number of industrial processes such as food processing, fermentation, textile, paper industries, and in biotechnology (2). These enzymes are produced by a variety of micro-organisms such as *Aspergillus niger*, *A. awamori*, *A. oryzae*, *A. tamarii*, *Bacillus subtilis*, *B. licheniformis*, *Rhizopus oryzae*, *Candida guilliermondii* and *Thermomyces lanuginosus* (2-7).

Among them *A. niger*, *B. subtilis* and *R. oryzae* are the most applied species in industry (2, 4-5).

Likewise, invertase is one of the most important commercial enzymes used in the food industry. It catalyzes the hydrolysis of sucrose in an equimolar mixture of glucose and fructose, known as sugar invert (8). Invertase can be produced by various microorganisms, among them, *S. cerevisiae*, *C. guilliermondii*, *T. lanuginosus*, *A. niger*, *A. flavus*, *A. japonicus*, *A. ochraceus*, *Penicillium chrisogenum* and *B. macerans* are considered as suitable producers of invertases (9-16).

For industrial production of these enzymes, it is imperative to find inexpensive inducers among the non-conventional substrates. The biosynthesis of these enzymes from energy crops, municipal solid wastes, agricultural and forest residues, or

other forms of lignocellulosic biomass could improve the economy of α-amylase and invertase production (2, 8).

The aim of the present study was to investigate the potential of using date wastes as a substrate for the production of α-amylase and invertase using *A. niger* strain ANSS-B5, which was isolated from saline soil areas of the Algerian sahara, and to be able to optimize their production conditions.

2. Materials and Methods

2.1. Materials

The date waste were obtained from Deglet-Nour cultivar.

2.2. Biological strains

2.2.1. Isolation, identification and screening of A. niger

Thirty strains of *A. niger* were isolated from the saline soils collected from five arid locations in Algeria. The strains were then seeded into Petri plates containing in: sucrose 30g/L, Na_2NO_3 2.0 g/L, KH_2PO_4 1.0 g/L, $MgSO_4$ 0.5 g/L, KCl 0.5 g/L, $FeSO_4$ 0.01 g/L and agar 20.0 g/L. After incubation at 30°C for 3 to 5 days, the mycelium was sub-cultured into 2 % water agar for purification (17). The identification of strains of *A. niger* was based on the observations of the fungal mycelium: color and diameter of the colony, the presence of the thallus, the presence or the absence of the septum, the nature of the production and characteristics of fruiting bodies and spores (18). Finally, the strains were screened qualitatively in petri plates containing Czapek-Dox agar medium with Bromocresol green as an indicator (17).

2.3. Methods

2.3.1. Experimental protocol

2.3.1.1. Preparation of culture substrate

The preparation of the date wastes syrup was reported by Acourene and Ammouche (19).

2.4. Inoculum preparation

A. niger strain ANSS-B5 was inoculated in sterile 100 ml Erlenmeyer flasks containing 20 ml of culture medium composed by: Glucose, 20 g/L; $(NH_4)\ 2SO_4$, 6.6 g/L ; KH_2PO_4, 3.5 g/L; $FeSO_4.7H_2O$, 0.15 g/L; $MgSO_4.7H_2O$, 0.10 g/L; $MnCl_2.2H_2O$, 0.45 g/L and mycological peptone, 3.0 g/L; at pH 6.0. The culture was incubated at 30°C, shaking at 220 rpm/min for 48 h at pH 5.0 (5, 20).

2.5. Production of α-amylase

The cultures were developed in 250 ml Erlenmeyer flasks containing, 50 ml of date waste syrup inoculated with 2% (w/v) inoculum level of 2×10^8 CFU/ml and shaking at 200 rpm/min.

2.6. Production of invertase

The fermentation was carried out in 250 ml Erlenmeyer flasks containing 50 ml of date wastes syrup inoculated with 2% (w/v) inoculum level of 2×10^8 CFU/ml and shaking at 200 rpm/min.

2.7. Analytical Methods

Biomass was harvested by paper filtration using a pre-dried and pre-weighted Whatman filter paper N°1, washed with distilled water and dried to a constant weight at 70°C (5).

Amylase activity was assayed by adding 0.1 ml of enzyme fermented broth supernatant to 0.2 ml of 0.5 % soluble starch and incubated for 30 min at 37°C. The reaction was stopped by adding 0.4 ml of 3,5 dinitrosalicylic acid, followed by boiling for 10 min. The final volume was made to 10 ml with distilled water and the absorbency measured at 540 nm with U.V. spectrophotometer. A calibration curve of absorbance and concentration of D-glucose was established with a known amount of glucose (21).

Invertase activity was determined by using the method of Sumner and Howells (22) with slight modification by incubating 0.1 mL of the enzyme solution with 0.9 ml of sucrose in 0.03 M acetate buffer (pH 5.0). To stop the reaction, 1 ml of dinitrosalicylic acid reagent was added and heated for 5 min in a boiling water bath. The absorbance was read at 540 nm in U.V spectrophotometer (23).

3. Results

3.1. Production of α-amylase and invertase

Thirty strains of *A. niger* isolated locally were tested for their potential to produce α-amylase and invertase. While all strains in the screening experiment were able to grow on date wastes syrup and were capable of producing α-amylase and invertase, with *A. niger* strain ANSS-B5 was

selected as the best producer of these enzymes, thus its production conditions were optimized.

3.2. Optimisation of α-amylase production

The obtained results indicate that dry biomass and α-amylase production were 0.98 g/L and 72.06 U/ml at 48 h and increased to 1.74 g/L and 143.33 U/ml at 96 h (Table 1).

Beyond 96 h, a decrease in α-amylase activity, 131.07 U/mL was noted at 144 h. To evaluate the effect of temperature on α-amylase production the results show that the dry biomass and α-amylase produced at 20°C are low, 0.78 g/L and 100.37 U/mL, and increased to a maximum of 1.74 g/L and 143.33 U/mL, respectively at 30°C. At higher temperatures there were gradual decreases in dry biomass and α-amylase production, 0.42 g/L and 24.50 U/mL when temperature was increased to 45°C.

As shown in Figure 1, the highest levels of dry biomass and α-amylase activity, i.e., 2.07 g/L and 176.05 U/ml were produced at pH 5.5, and their lowest levels, i.e., 0.96-1.18 g/L, and 95.67-126.00 U/mL, were obtained at pH 4.0 and 9.0, respectively.

On the other hand, the obtained results show that the growth and α-amylase activity increases gradually with an increase in sugars content to reach to maximum values at 20.0 g/L of sugars, 2.38 g/L and 216.27 U/mL, and their lowest levels, 1.65 g/L and 107.85 U/mL, were obtained at 2.5 g/L of sugars.

Other results obtained elsewhere show that the highest dry biomass production was obtained with yeast extract, 2.96 g/L followed by urea, 2.38 g/L, ammonium nitrate, 2.26 g/L, peptone, 2.12 g/L, meat extract, 1.88 g/L, Gunpowder milk, 1.86 g/L, Ammonium phosphate, 1.84 g/L, Ammonium sulfate, 1.79 g/L and ammonium carbonate, 1.48 g/L. Among the organic sources, supplementation with yeast extract and urea caused an increase in α-amylase activities, up to 256.27 and 216.26 U/mL, respectively. Ammonium nitrate also enhanced the amylase activity 200.07 U/mL, but ammonium carbonate showed a negative influence, showing a steep decrease in α-amylase activity, 108.36 U/mL.

As shown, in Figure 2, the growth increased with an increase in yeast extract content to reach to a maximum value, 4.12 g/L at 5.0 g/L.

The α-amylase activity also reached the maximum value, 273.37 U/mL at 5.0 g/L of yeast extract. However, beyond 5.0 g/L yeast extract, the α-amylase activity dropped to 216.53 U/mL.

Finally, the obtained results show that the growth increased gradually with increased concentration of potassium phosphate reaching to a maximum values of 3.62-3.84 g/L at 6.0-8.0 g/L of potassium phosphate. The α-amylase activity maximum values, 273.37-288.26 U/ml, were obtained with potassium phosphate content ranging between 4.0 to 8.0 g/L. The lowest growth and α-amylase activity, 1.82 g/L and 125.47 U/mL, respectively were obtained at 2.0 g/L of potassium phosphate content.

Table 1. Evaluation of dry biomass and α-amylase activity following incubation period, temperature, sugars and potassium phosphate content

Incubation period	Time in h	48	72	96	120	144
	Dry biomass	0.98	1.12	1.74	1.94	2.00
	α-amylase activity	72.06	137.00	143.33	142.31	131.07
Temperature	°C	20	30	35	40	45
	Dry biomass	0.78	1.74	1.36	0.74	0.42
	α-amylase activity	100.36	143.33	111.53	80.00	24.50
Sugars	Content in g/L	2.5	5	10	15	20
	Dry biomass	1.65	1.74	1.98	2.07	2.38
	α-amylase activity	107.85	129.80	161.23	176.05	216.27
Potassium phosphate	Content in g/L	2.0	4.0	5.0	6.0	8.0
	Dry biomass	1.82	3.29	3.36	3.62	3.84
	α-amylase activity	125.47	273.37	285.60	288.26	287.80

3.3. Optimisation of invertase production

The results obtained show that the growth and invertase production were 2.60 g/L and 25.62 U/mL at 48 h and increased up to 3.60 g/L and 124.32 U/mL at 96 h (Table 2).

Interestingly, after 96 to 144 h, growth increased slightly, 5.26 g/L at 144 h, but a lower invertase activity was observed, 109.88 and 97.66 U/ml at 120 h and 144h, respectively. Temperature was varied to evaluate its effect on invertase production. The results show that the maximum growth and invertase activity, i.e., 3.60 g/L and 124.32 U/mL, were obtained at 30°C. Further increase in temperature declines the growth and invertase production, 1.82 to 2.54 g/L, and 32.34 to 65.61 U/mL at temperatures of 45 and 40°C, respectively.

As shown in Figure 3, the growth and invertase activity were at maximum at initial pH 5.0 and pH 6.0, 3.60-3.75 g/L and 124.32 - 133.59 U/mL, respectively. The lowest invertase activity, 93.58 U/mL was obtained at pH 3.0. At pH 7.0, dry biomass and invertase production declined, i.e., 2.40 g/L and 111.14 U/mL, respectively.

The results obtained show that the growth and invertase activity increased gradually with sugars content to reach to maximum values of 3.95-4.76 g/L and 170.10-171.20 U/mL, respectively at 40.0 -50.0 g/L of sugars content. The lowest values, 2.40 g/L and 27.32 U/mL, respectively were obtained at 10.0 g/L of sugars.

Yeast extract content was varied to evaluate the effect on invertase production. As shown in Figure 4, the higher invertase production, 195.56 U/ml was obtained at 11.0 g/L of yeast extract content and the dry biomass was 4.22 g/L.

The lowest values, 0.20 to 2.80 g/L and 0 to 62.33 U/mL were obtained at yeast extract contents ≤ 6.0 g/L. At higher yeast extract contents ≥ 12.0 g/L, a decrease in invertase production, 163.59 U/ml but an improvement of growth, 5.20 g/L, were noted.

The effect of potassium phosphate content on invertase production was measured.

The results show that the addition of potassium phosphate to the culture medium improved the growth of *A. niger* and the maximum dry biomass, 4.65 g/L at 4.0 g/L of potassium phosphate. Whereas, the maximum invertase production, 195.56 U/mL was obtained at 3.5 g/L. Any decrease in the phosphate concentration beyond optimum, greatly reduced the invertase activity due to improper growth of *A. niger* ANSS-B5, thus the lowest growth and invertase activity, 2.10 g/L and 60.15 U/mL, respectively were obtained at 1.0 g/L of potassium phosphate content.

4. Discussion

4.1. Optimization of α-amylase production

The incubation time for achieving maximum enzyme level is governed by the characteristics of the culture. So, the optimum fermentation period for maximum α-amylase production was 96 h. On further incubation, the enzyme activity gradually decreased. This may be due to the depletion of

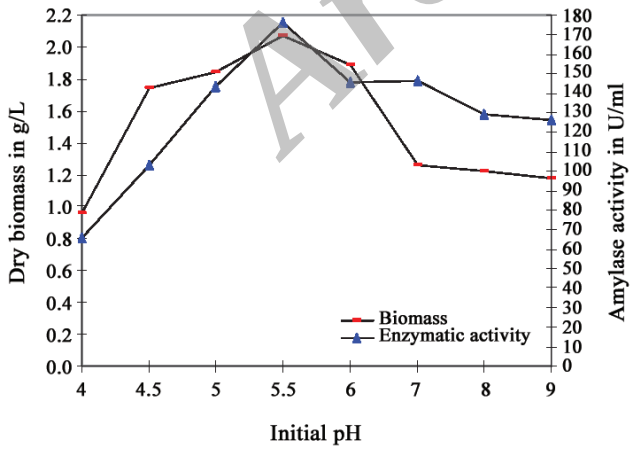

Figure 1. Evaluation of dry biomass and α-amylase activity following initial pH after 96 h of incubation, temperature of 30°C, sugars content of 15 g/L, urea as a source of nitrogen at 4.0 g/L and potassium phosphate content of 4.0 g/L

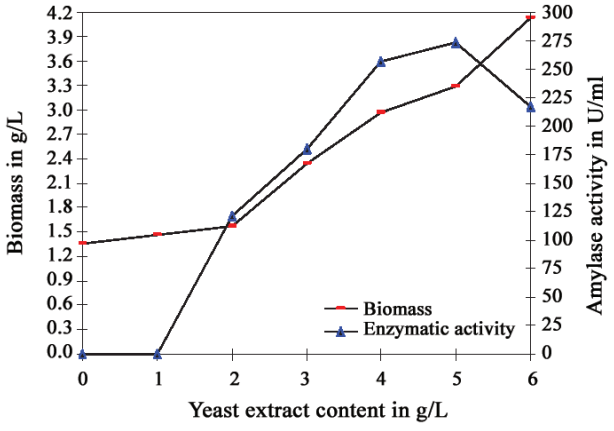

Figure 2. Evaluation of dry biomass and α-amylase activity following yeast extract content after 96 h of incubation, temperature of 30°C, initial pH 5.5 and potassium phosphate content of 4.0 g/L.

Table 2. Evaluation of dry biomass and invertase activity following incubation period, temperature, sugars and potassium phosphate content

Incubation period	Time in h	48	72	96	120	144
	Dry biomass	2.60	3.20	3.60	4.80	5.26
	Invertase activity	25.62	68.80	124.32	109.88	97.66
Temperature	°C	20	30	35	40	45
	Dry biomass	2.73	3.60	2.84	2.54	1.82
	Invertase activity	92.54	124.32	91.22	65.61	32.34
Sugars	Content in g/L	10	20	30	40	50
	Dry biomass	2.40	3.00	3.75	3.95	4.76
	Invertase activity	27.32	103.2	133.59	170.12	171.20
Potassium phosphate	Content in g/L	1.0	2.0	3.0	3.5	4.0
	Dry biomass	2.10	3.05	4.16	4.22	4.65
	Invertase activity	60.15	115.36	170.20	195.56	194.66

essential nutrients required for the growth and enzyme production. Similar results were reported by Djekrif-Dakhmouche *et al*., (3); Guillen-Moreira *et al*., (4); Wang *et al*. (24). In contrast, Bhadwaj *et al*., (25); Farid and Shata (26); Suganthi *et al*., (27) have obtained higher α-amylase activity at 120 to 144 h with strains of *A. flavus*, *A. oryzae* LS1, *A. oryzae* NRRL 6586 and *A. niger* BAN-3E.

Temperature is one of the important factors, which strongly affects the submerged fermentation (4). The optimum temperature for maximum α-amylase activity was 30°C, and Djekrif-Dakhmouche *et al*. (3); Ramachandran *et al*. (6); Acourene and Ammouche (19); Wang *et al*. (24); Bhardwaj *et al*. (25); Alva *et al*. (28); Gupta *et al*. (29); Jin *et al*. (30); Renato *et al*. (31); Reazaei *et al*. (32) have also reported similar results. A further increase in temperature resulted in a decrease in α-amylase activity. At temperature above 45°C resulted in moisture loss of the substrate, which affects metabolic activities of the micro-organism and resulted in reduced growth and α-amylase production (7). In contrast, Ray *et al*. (33) reported that the optimum temperature for maximum α-amylase production by *B. brevis* is 50°C.

Among the physicals parameters, the pH of medium plays an important role by inducing morphological changes in the organism and in enzyme secretion (29). The production of α-amylase is

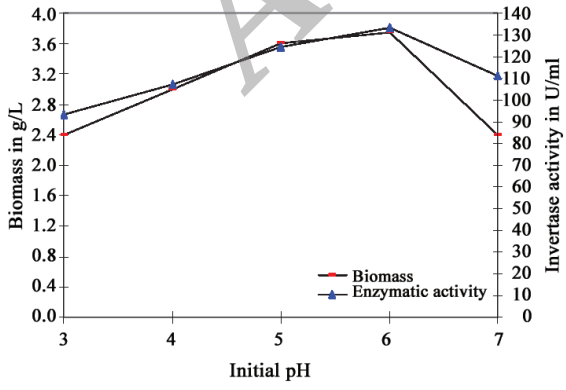

Figure 3. Evaluation of dry biomass and invertase activity following initial pH after 96 h of incubation, temperature of 30°C, sugars content of 30.0 g/L, yeast extract content of 10.0 g/L and potassium phosphate content of 3.0 g/L

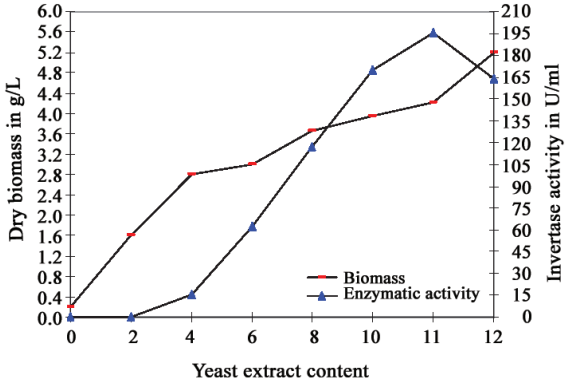

Figure 4. Evaluation of dry biomass and invertase activity following yeast extract content after 96 h of incubation, temperature of 30°C, initial pH 6.0, sugars content of 40.0 g/L and potassium phosphate content of 3.0 g/L

very sensitive to initial pH of the medium. Our findings are comparable to previously reported results from literature that reported maximum α-amylase production at a pH varying between 5.0 and 6.0 (3, 7, 19, 25, 28-30, 32). According to Varalakhshmi *et al*., (34), the amylases production by *A*. *flavus* and *A*. *niger* JGI 24 were found maximum at pH 7.0 to 7.5.

In contrast, Gangadharan *et al*., (35) reported that pH 3.5 to be the best for the production of á-amylase. According to Guillen-Moreira *et al*., (4); Gupta *et al*., (29), the growth and enzyme production were inhibited when the initial pH of the medium was above 10.0 or below 4.0.

Sugar content is a crucial factor in submerged fermentation and its importance for α-amylase production has been well established. The optimum sugars content for maximum yield was 20.0 g/L. Similar results were obtained by Hernandez *et al*., (5) and Acourene and Ammouche (19) with strains of *C*. *guilliermondii* CGL-A10 and *A*. *niger*.

Studies on supplementation of inorganic and organic nitrogen sources to the date wastes syrup showed a mixed trend on enzyme production. Added nitrogen sources have been reported to have an inducing effect on the production of various enzymes including α-amylase (36). This study shows that the yeast extract is the best nitrogen source. Similar results were reported by Hernandez *et al*., (5); Bhardwaj *et al*., (25); Anto *et al*., (37) with *A*. *niger* and *A*. *flavus*. According to Ray *et al*., (33); Varalakshmi *et al*., (34), *B*. *brevis* and *A*. *niger* JGI 24 produced more amylase in the presence of meat extract or beef extract as nitrogen source.

In contrast, Hernandez *et al*., (25); Gupta *et al*. (29) showed that the peptone was the best nitrogen source. Finally, Radha-Krishna *et al*., (38) obtained high amylase activity when ammonium nitrate was used.

On the other hand, the optimum yeast extract content for maximum α-amylase production was 5.0 g/L. Similarly, Guillen-Moreira *et al.* (4) obtained at a organic nitrogen source of 5.0 g/L. Moreover, the maximum gluco-amylase produced was obtained with a concentrations of yeast extract ranging between 3.0 and 5.0 g/L (39), whereas Rezaei *et al*., (32) obtained high α-amylase activity with *A*. *niger* at 2.0 g/L the organic nitrogen source. In contrast, Radha-Krishna *et al*., (38) obtained a maximum α-amylase activity at 10.0 g/L of ammonium nitrate as inorganic nitrogen source with *A*. *niger*.

Phosphate serves as the construction material of cellular components such as cyclic AMP, nucleic acids, phospholipids, nucleotides and coenzymes (35). The optimum phosphate content for maximum growth and α-amylase production was 5.0 g/L. Similar results were recorded by Acourene and Ammouche (19); Gangadharan *et al*. (35) with *C*. *guilliermondii* and *B*. *amyloliquefaciens*.

Moreover, Zaldivar-Aguero *et al*., (40) reported that the synthesis of α-amylase is stimulated by adding 7.0 to 10.0 g/L phosphorus to the culture medium. In contrast, Jin *et al*., (30) did not obtain a significant improvement in gluco-amylase yields by adding phosphorus to the fermentation medium.

4.2. Optimization of invertase production

The incubation period is governed by the characteristics of the culture and is based on the growth rate and enzyme production. In this study, the optimum incubation period for maximum invertase production was 96 h. A similar result was found by Ashokumar *et al*. (10); Cheng and Liu (12); Guimaraes *et al*. (13); Uma *et al*. (16); Praveen-Reedy *et al*., (41); Uma *et al*., (42) with *A*. *niger*, *A*. *japonicas*, *A*. *ochraceus*, *A*. *fumigatus* and *A*. *flavus*. Further increase in the incubation time caused a slow decrease in invertase activity. Similar results were reported by Praveen-Reedy *et al*., (41) and Gomez *et al*., (43).

The optimum temperature in the present study for maximum growth and biosynthesis of invertase was 30°C. Further increase in temperature caused a decline in the invertase production. Similar findings were recorted by Uma *et al*. (16); Praveen-Reedy *et al*. (41); Uma *et al*. (42); Alegre *et al*. (44); Rajoka and Yasmeen (45) with strains of *A*. *caespitosus*, *A*. *niger*, *A*. *fumigatus* and *A*. *flavus*. In contrast, Viana *et al*., (46) obtained high invertase activity at temperature of 40°C with strain of *A*. sp GH1.

Optimal pH is very important for growth of micro-organisms and their metabolic activities. In our system, an initial pH 6.0 was regarded as optimal for invertase production. A similar result was reported by Hayashi *et al*., (8); Cheng and Liu (12); Praveen-Reedy *et al*., (41); Alegre *et al*.,

(44); Rajoka and Yasmeen (45); Shankar *et al*. (47); Sirisansaneeyakul *et al*., (48) with strains of *A*. *caespitosus*, *A*. *japonicus* and *A*. *niger*. In contrast, Guimaraes *et al*., (13); Praveen-Reedy *et al*., (41); Viana *et al*., (46) obtained maximum invertase production with an initial pH ranging between 3.5 to 4.5 with strains of *A*. *ochraceus*, *A*. niger and Fusarium sp.

Sugars content influenced strongly the level of invertase production. We found the optimum sugars content for maximum invertase activity to be 40.0 g/L. This optimum was similar to the level reported by Ashokumar *et al*., (10) with *A*. *niger*. In contrast, Uma *et al*., (42) obtained maximum invertase production at 10.0 g/L of sugars content with *A*. *fumigatus*.

Nitrogen constituent has a profound effect on invertase production because there exists a strong correlation between nitrogen equilibrium and the productivity of microorganisms (14). We report the optimal yeast extract content for maximum invertase production is 11.0 g/L. A similar result was reported by Hayashi *et al*., (8). In contrast, Cheng and Liu (12) obtained a maximum invertase activity at 26.0 g/L of yeast extract with *A*. *japonicus*.

Phosphate source plays a key role in enhancing invertase secretion (43). The optimum potassium phosphate content for maximum invertase production was 3.5 g/L. Similar findings have previously been reported by Alegre *et al*., (44) with A. caespitosus. In contrast, according to Cheng and Liu (12), the addition of potassium phosphate shifted the morphology of the fungal growth from filamentous to pellet form, but this salt did not affect invertase production with *A*. *japonicus*.

5. Conclusions

α-Amylase and invertase are industrially important enzymes and their demand is increasing in line with the growing global markets for processed food, especially the confectionery industry. The use of α-amylase and invertase are somewhat limited due to its high price. Thus the optimization of the production process is very important, so as to make it more economical and feasible. The present study contributed toward to the optimization of nutritional parameters and cultural conditions.

The growth and α-amylase or invertase production with *A*. *niger* ANSS-B5 were influenced by various nutritional and environmental factors. The obtained results show that the composition of the medium is a major factor in regulating of the synthesis of α-amylase and invertase.

It is concluded from these results that *A*. *niger* ANSS-B5 isolated from saline soil of Algerian aridic regions when cultured in submerged fermentation using date wastes product as carbon source is very interesting for industrial scale applications in the production of α-amylase and invertase.

Acknowledgements

Acourene Said is thankful to the Algerian National Institute of Agricultural Research for financial assistance during this work.

References

1. Anonymous. Date palm, Areas and productions. In: *Agricultural statistics*, Series B, Agricultural Statistics Direction of the Algerian Agricultural Ministry (ASDAAM) ed., Alger. 2011;PP. 50-55.
2. Pandey A, Nigam P, Soccol C R, Soccol VT, Singh D, Mohan R. Advances in microbial amylases, *Biotechnol Appl Biochem*. 2000;**31**:135-152.PMID:10744959[PubMed - indexed for MEDLINE]
3. Djekrif-Dakhmouche S, Gheribi-Aoulmi Z, Meraihi Z, Bennamoun L. Application of a statistical design to the optimization of culture medium for amylase production by *Aspergillus niger* ATCC 16404 grown on orange waste powder. *Food Eng*. 2006;**73**:190-197.doi:10.1016/j.jfoodeng.2005.01.021
4. Guillen-Moreira F, Arrias de Lima F, Fazzano Pedrinho SR, Lenartovicz C, Giatti Marques de Souza RM, Peralta C. Production of amylases by *Aspergillus tamari*. *Revista Microbiologica*. 1999;**30**(2):1-9.
5. Hernandez MS, Rodrýguez MR, Perez-Guerra N, Perez-Roses R. Amylase production by *Aspergillus niger* in submerged cultivation on two wastes from food industries. *J Food Eng*. 2006;**73**: 93-100.doi:10.1016/j.jfoodeng.2005.01.009
6. Ramachandran S, Patel AK, Nampoothiri KM, Chandran S, Szakacs G, Soccol CR, Pandey A. Alpha amylase from a fungal culture grown on oil cakes and its properties. *Brazilian Archiv Biol Technol*. 2004;**47**:309-317.
7. Sindhu R, Suprabha GN, Shashidhar S. Optimization of process parameters for the production of α-amylase from *Penicillium janthinellum* (NCIM 4960) under solid state fermentation. *African J Microbiol Res*. 2009;**3**(9):498-503.
8. Hayashi A, Matsuzaki Y, Takasaki Ueno H, Imada K. Production of β-fructofuranosidase by *Aspergillus japonicus*. *World J Microbiol Biotechnol*. 1992;**8**:155-159.doi:10.1007/BF01195837
9. Aranda C, Robledo A, Loera O, Contreras-Esquivel JC,

Rodriuez R, Aguilar N. Fungal invertase expression in solid state fermentation. *Food Technol Biotechnol*. 2006;**44**(2):229-233.

10. Ashokkumar B, Kayalvizhi N, Gunasekaran P. Optimization of media for β-fructofuranosidase production by *Aspergillus niger* in submerged and solid state fermentation. *Process Biochem*. 2001;**37**(4): 331-338.
11. Bousmaha L, El-Moualidi L, Oouhssine M, El-Yachioui M. Souches de *Candda guilliemondii* isolées de la saumure de carottes productrices d'une β-Fructofuranosidase extracellulaire. *Bulletin Societé Pharm*acie. 2007;**146**:51-62.
12. Cheng WC, Liu C. Production of β-fructofuranosidase by *Aspergillus japonicus*. *Enzyme Microb Technol*. 1996;**18**(2):153-160.doi:10.1016/0141-0229(95)00099-2
13. Guimaraes LHS, Terenzi HF, Polizeli ML, Jorge JA. Production and characterization of a thermostable extracellular β-D-fructofuranosidase produced by *Aspergillus ochraceus* with agro industrial residues as carbon sources. *Enzyme Microb Technol*. 2007;**42**:52-57.doi:10.1016/j.enzmictec.2007.07.021
14. Ikram UH, Mirza AB, Sikander A. Effect of cultivation conditions on invertase production by hyperproducing *Saccharomyces cerevisiae* isolates. *World J Microbiol Biotechnol*.2005;**21**:487-492.doi:10.1016/j.enzmictec.2007.07.021
15. Kotywal SM, Shankar V. Immobilised invertase. *Rev Biotechnol Adv*. 2009;**27**:311-322.doi:10.1007/s11274-004-2612-7
16. Uma C, Gomathi D, Muthulakshmi C, Gopalakrishnan VK. Production, Purification and characterization of invertase by *Aspergillus flavus*using fruit peel waste as substrate. *Adv Biol Res*. 2010a;**4**(1):31-36.
17. Botton B, Bretton A, Fever M, Gautier S, Guy PH, Larpent JP, Reymond P, Sanglier JJ, Vayssier Y, Veau P. *Moisissures utiles et nuisibles, importance industrielle*. Masson, Paris;1990.
18. Hawksworth DL, Kirk PM, Sutton B, Pegler DN. *Ainsworth and Bisby's Dictionary of the Fungi*. Commonwealth Mycological Institute, CABI publishing, London;1995.
19. Acourene S, Ammouche A. Optimization of ethanol, citric acid, and α-amylase production from date wastes by strains of *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Candida guilliermondii*. *J Ind Microbiol Biotechnol*. 2012;**39**(5):759-766.doi:10.1007/s10295-011-1070-0
20. Romero-Gomez S, Augur C, Viniegre-Gonzalez G. Invertase production by *Aspergillus niger* in submerged and solid-state fermentation. *Biotechnol Letters*. 2000;**22**:1255-1258.
21. Bernfeld P. Amylase, α/β. In: *Methods in Enzymology*, Academic Press ed., New-York. 1955;PP. 149-154.
22. Sumner JB, Howell SF. A method for determination of saccharase activity. *J Biol Chem*. 1935;**108**:51-54.
23. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem*. 1959;**31**:426-428.
24. Wang Q, Wang X, Maa H. Glucoamylase production from food wastes by *Aspergillus niger* under submerged fermentation. *Process Biochem*. 2008;**43**:280-286.doi:10.1016/j.procbio.2007.12.010
25. Bhardwaj S, Bhattacharya S, Anand S, Das A. Production and characterization of amylase from a mangrove isolate of *Aspergillus flavus* using sugar cane bagasse in solid state fermentation. *American Euras J Agr Environ Sci*. 2011;**3**(4):171-181.
26. Farid MAF, Shata HAH. Amylase production from *Aspergillus oryzae* LS1 by solid-state fermentation and its use for the hydrolysis of wheat flour. *Iranian J Biotechnol*. 2011;**9**(4):267-274.
27. Suganthi R, Benazir JF, Santhi R, Ramesh K, Hari A, Meenakhi N, Nidhiya KA, Kavitha G, Lakshmi R. Amylase production by *Aspergillus niger* under solid state fermentation using Agro- industrial wastes. *Int J Eng Sci Technol*. 2011;**3**(2):1756 1763.
28. Alva S, Anupama J, Savla J, Chiu YY, Vyshali P, Shruti M, Yogeetha BS, Bhavya D, Purvi J, Ruchi K, Kumudini, BS, Varalakshmi KN. Production and characterization of fungal amylase enzyme isolated from *Aspergillus sp*. JGI 12 in solid state culture. *African J Biotechnol*. 2007;**6**(5):576-581.
29. Gupta A, Gautam N, Modi, DR. Optimization of amylase production from free and immobilized cells of *Aspergillus niger*. *J Biotechnol Pharm Res*. 2010;**1**(1):1-8.
30. Jin B, Van-Leeuan HJ., Patel B, Yu Q. Utilization of starch processing wastewater for production of microbial biomass protein and fugal α-amylase by *Aspergillus oryzae*. *Bioresour Technol*. 1998;**66**:201-206.doi:10.1016/S0960-8524(98)00060-1
31. Renato PR, Nelson PG. Optimization of amylase production by *Aspergillus niger* in solid-state fermentation using sugarcane bagasse as solid support material. *World J Microbiol Biotechnol*. 2009;**25**(11):929-1939.doi:10.1007/s11274-009-0091-6
32. Rezaei PS, Darzit GN, Shafaghat H. Optimization of the fermentation conditions and partial characterization for acido-thermophilic α-amylase from *Aspergillus niger* NCIM 548. *Korean J Chem Eng*. 2010;**27**(3):919-924.doi:10.1007/s11814-010-0138-2
33. Ray RC, Kar S, Nayak S, Swain MR. Extracellular α-amylase production by *Bacillus brevis* MTCC 7521. *Food Biotechnol.* 2008;**22**:234-246.doi:10.1080/08905430802262558
34. Varalakshmi KN, Kumudini BS, Nandini BN, Solomon J, Suhas R, Mahesh B, Kavitha AP. Production and characterization of α-amylase from *Aspergillus niger* JGI 24 isolated in Bengalore. *Polish J Microbiol*. 2009;**58**(1):29-36.
35. Gangadharan D, Sivaramakrishnan S, Nampoothiri KM, Pandey A. Solid culturing of *Bacillus amyloliquefaciens* for α-amylase production. *Food Technol Biotechnol*. 2006;**44**(2):269-274.

36. Kaur P, Grewal HS, Kocher GS. Production of α-amylase by *Aspergillus niger* using wheat bran in submerged and solid state fermentations. *Indian J Microbiol*. 2003;**43**:143-145.
37. Anto H, Trivedi UB, Patel KC. Glucoamylase production by solid-state fermentation using rice flake manufacturing waste products as substrate. *Bioresour Technol*. 2006;**97**(10):1161-1166.doi:10.1016/j.biortech.2005.05.007
38. Radha-Krishna P, Srivastava AK, Ramaswamy NK, Suprasanna SF, D'Souza P. Banana peel as substrate for α-amylase production using *Aspergillus niger* NCIM 616 and process optimization. *Indian J Biotechnol*. 2012;**11**:314-319.
39. Kunameni A, Permaul K, Singh S. Amylase production in solid-state fermentation by the thermophilic fungus *Thermomyces lanuginosus*. *J Biosci Bioeng*, 2005;**100**: 168-171.doi:10.1263/jbb.100.168
40. Zaldivar-Aguero JM, Badino AC, Vilaça PR, Facciotti MCR. Schmide W. Influence of phosphate concentration on gluco-amylase production by *Aspergillus awamori* in submerged culture. *Brazilian J Chem Eng*. 1997;**14**:104-108.
41. Praveen-Reddy P, Reddy GSN, Sulochana MB. Highly thermostable β-fructofuranosidase from *Aspergillus niger* PSSF21 and its application in the synthesis of fructooligosacharides from agro industrial residue. *Asian J Biotechnol*. 2010;**2**:86-98.doi:10.3923/ajbkr.2010.86.98
42. Uma C, Gomathi D, Muthulakshmi C, Gopalakrishnan VK. Optimization and characterization of invertase by *Aspergillus fumigatus* using fruit peel waste as substrate. *Res J Pharm Biol Chem Sci*. 2010b;**1**(2):93-101.
43. Gomez SJR, Augur C, Viniegre-Gonzalez G. Invertase production by *Aspergillus niger* in submerged and solid-state fermentation. *Biotech Lett*ers. 2000;**22**: 1255-1258.
44. Alegre ACP, Teixeira de Moraes-Polizeli, ML, Terenzi HF, Jorge JA, Souza-Guimaraes LH. Production of thermostable invertase by *Aspergillus caespitosus* under submerged or solid state fermentation using agro-industrial residues as carbonate source. *Brazilian J Microbiol*. 2009;**40**: 612-622.
45. Rajoka MI, Yasmeen A. Improved productivity of β-fructofuranosidase by a depressed mutant of *Aspergillus niger* from conventional and non-conventional substrates. *World J Microbiol Biotechnol*. 2005;**21**(4):471-478.doi:10.1007/s11274-004-1995-9
46. Veana F, Aguilar CN, Rodríguez Herrera R. Kinetic studies of invertase production by xerophilic *Aspergillus* and *Penicillium* strains under submerged cultures. *Micologia Aplicada Int*. 2011;**23**(2):37-45.
47. Shankar S, Madhan R, Sathyvani R, Niket B. Production and partial purification of invertase using *Cympopogan caecius* leaf powder as substrate. *Indian Microbiol*. 2010;**50**(3):318-324.doi:10.1007/s12088-010-0011-3
48. Sirisansaneeyakul S, Jitbanjongkit S, Prasomsart N, Luangpituksa P. Production of β-fructofuranosidase from *Aspergillus niger* ATCC20611. kasestart *J Nat Sci*. 2000;**34**:378-386.