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Optimization of Bakery Yeast Production Cultivated on Musts of Dates

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Abstract: The objective of this study is the use of dates like substrate for the production of saccharomyces cerevisiae. The study of the kinetics of growth of four strains of Saccharomyces cerevisiae shows that SDB strain gives the best results to know, a generation time reduced, a high growth rate and a high quantity of biomass. The results obtained on fermentation in Fed-batch show that date musts and more particularly those of the offal's of Deglet-Nour and Tinissine give yields in biomass raised compared to the medium of fermentation containing molasses. Nevertheless, the enrichment of these musts with nitrogen, phosphorus and vitamins is necessary in order to improve the yield in biomass and the force of levy. For this purpose, the use of the sulphate of ammonia and urea with 50 - 50% improve of more than 36%, the yield in biomass compared to urea. On the other hand, the use of ammonium phosphate improves it from 38 to 55%. As regards the vitamin source, it is not necessary to bring vitamins during fermentation in spite of a light improvement of the yields in biomass is more than 6% by adding 0.6mg/l of thiamin.

Key words: Musts of dates/ Fermentation/ *Saccharomyces cerevisiae*/ Strain/ Biomass

INTRODUCTION

The agricultural activities and food industry generate some important quantities of waste rich in organic matter who could constitute new materials for a lot of industry. To this effect, their valorization by the biotechnical processes represents a solution of choice insofar as it allows producing some substances to high added value. In Algeria, the production of dates is estimated to 437 000 tons which 60 000 to 75 000 tons is minus appreciated on the market, constituted of common of dates and offal's of Deglet-Nour[1]. By elsewhere, he exists in Algeria two farmers of production of bakery yeast using more than 20 000 tons of molasses by year[2]. However, the utilization of the molasses of beet or of cane could cause some constraints because they can contain some inhibiting of fermentation such as pesticide used during the culture of beets or of cane[3].

By elsewhere, with the improvement of process of extraction of the sugars used by the sweetmeats, a decrease of the content in sugars of molasses is noted.

This study has objectives: The utilization of the offal's of Deglet-Nour and some varieties of common dates as the substrate for the production of *saccharomyces cerevisiae*. Indeed, these dates are rich in sugars that could be used as carbonaceous source of fermentation for the production of the biomass. In this sense, the utilization of the date as means of substitution to the molasses is justified because she is produced locally in grand quantity and to inexpensive.

Nevertheless, the fixation of the conditions of fermentation and the optimization of the parameters of production of *Saccharomyces cerevisiae* cultivated on substrate at basis of dates is necessary in order to pronounce.

MATERIALS AND METHODS

1/ Material

1.1/Vegetable Material: The vegetable material used is constituted of some Offal's of dates of Deglet-Nour and the dates produced by Tinissine and Tantboucht.

1.2/ Biologic Material:

The biologic material used is constituted of four strains of *Saccharomyces Cerevisiae*:

ATCC 1102 (Derived from the industrial bakery yeast) and the strains isolated from some varieties of dates and are named, SDB (strain isolated from Degla-

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Beida), SDN (strain isolated from Deglet-Nour) and STB (strain isolated from Tantboucht).

2/ Experimental Protocol

2.1/ Preparation of Culture Media or must of Dates: The dates are washed themselves, destoned and grounded. Two and 1/2 liters of hot water at 80-85°C were added to 1 kg of date, homogenized and filtered through a cloth. The pH of juice is fixed between 4.3-4.7 and sterilized to 120°C during 20 minutes[4].

2.2/ Production of *Saccharomyces cerevisiae* in Batch Fermentation

Preparation of the Inoculums: A stock culture of *Saccharomyces cerevisiae* was maintained on agar media slopes which were stored in screw-cap bottles at 4°C. Sub-culturing was carried out at intervals of one month. The inoculums were prepared by transferring yeast cells from slant of 250 ml of Carlsberg medium. The flasks were subjected to the orbital previously adjusted at 30°C and 150 rpm and incubated for 18 hours[4].

Batch Fermentation: The inoculation is carried out by adding 20 ml of prepared inoculums for one liter of medium of culture having content in sugars of 2 % and enriched in nutritious elements following:

- 2.65 (g) of ammonium sulfate $[(NH_4)_2\ SO_4]$.
- 6.35 (g) of urea.
- 2.4 (g) of ammonium phosphate $[(NH_4)_2\ PO_4]$.

The temperature is fixed at 30°C and under continuous agitation. We instill the pure air in order to oxygenate the medium of fermentation and evacuating the CO2 in the same time produced by the metabolism of the carbonaceous substrates. The period of the fermentation is about 18 to 24 hours[5].

2.2/ Production of *Saccharomyces cerevisiae* in Fed-Batch Fermentation

Preparation of the Inoculums: A stock culture of *Saccharomyces cerevisiae* was maintained on agar media slopes which were stored in screw-cap bottles at 4°C. Sub-culturing was carried out at intervals of one month. The inoculums were prepared by transferring yeast cells from slant of 250 ml of Carlsberg medium. The flasks were subjected to the orbital previously adjusted at 30°C and 150 rpm and incubated for 18 hours [4].

Alcoholic Fermentation: We inoculate 300 ml of must of dates enriched in proteins and in mineral salts with 20 ml of prepared inoculums. The anaerobic fermentation steel 18 hours at a temperature of 30°C and under continuous agitation [2and 6].

Fed-Batch Fermentation: The Fed-Batch culture is unrolled on a period of 15 hours and we used a reactor having a capacity of three liters provided with all the accessories and is filled to the 2/3 of its volume. The temperature of fermentation is maintained constant to 30°C and the pH fixed at 4.5. Agitation is of 300 turns per minute and ventilation fixed at 2 V.V.M. The rate of alimentation of the reactor on substrate is regulated so that the concentration in this last is constant in the tank and to correspond to the phase logarithmic curve of cell multiplication [2 and 6].

3/ Analytical Methods

3.1/ Biochemical Analysis: The content in water is determined by drying 10 ml of juice at 105°C during 18 hours[7]. The content in ashes is determined by incineration one gram of juice at a temperature of 600°c during 3 hours[7]. The reducing sugars, the sucrose and the total sugars were determined by the method of Bertrand, reported by[8]. The total nitrogen is determined by the method of Kjedahl [8].

The mineral salts are determined according to the methods advocated by [9 and 10].

3.2/ Kinetic of Growth: We take off every two hours until 24 hours 10 ml of medium of fermentation and this intake of test is diluted to the 1/20. We make a reading in a spectrophotometer to a length of wave 620 nm (in absorbance). In this way there, we could determine, the latency phase, the exponential phase of growth, the rate of growth and time of generation [11].

3.3/ Analysis of Bakery Yeast

Quantity of Biomass: At the end of fermentation, the culture was harvested using centrifugation at 3500 rpm during 15 minutes to determine the fresh and dry weight in biomass and to provide a clear supernatant which was used for the determination of the residual sugar[12].

Residual Sugars: The content of residual sugars was determined by the method of Bertrand, reported by [8].

The Strength of Levy: She represents the volume of CO2 free by the yeast in bakery dough of given composition during a time determined to a given temperature.

The used method is the one of S.J.A who consists in determine the time of fermentation of the yeast and then the quantity of CO2 free in a table of correspondence [13].

RESULTS AND DISCUSSIONS

1/Biochemical Composition of Musts of Dates and Molasses: The obtained results shows that the musts of dates present an elevated in content of total sugars variable between 21.2 and 22.9 % of M.F

Table 1: Biochemical composition of musts of dates and molasses

Constituents	Offal's of D.N	Tantboucht	Tinissine	Molasses
Content in water in %	71.00	70.00	70.15	65.00
Reducing sugars in % of M.F	9.13	21.20	22.90	1.00
Sucrose in % of M.F	12.80	0	0	19.95
Total sugars in % of M.F	22.61	21.20	22.90	22.00
Proteins in % of M.F	0.24	1.05	0.80	1.00
Ashes in % of M.F	1.19	1.49	1.34	4.00
Sodium in mg/100 ml of M.F	295.00	225.00	230.00	1300.00
Potassium in mg/100 ml of M.F	260.00	520.00	480.00	1750.00
Calcium in mg/100 ml of M.F	280.00	230.00	210.00	75.00
Magnesium in mg/100 ml of M.F	45.00	70.00	65.00	5.00
Phosphor in mg/100 ml of M.F	14.25	37.00	33.00	30.00
Zinc in mg/100 ml of M.F	0.25	0.50	0.20	0.24
Copper in mg/100 ml of M.F	0.07	0.25	0.12	0.09
Iron in mg/100 ml of M.F	2.69	5.86	2.2	0.095
Manganese in mg/100 ml of M.F	0.07	0.14	0.08	0.03

Table 2: Needs in nutritious elements of *Saccharomyces cerevisiae*

Nutritious elements	Needs of *Saccharomyces cerevisiae*
Phosphor	2.20 - 3.60 g/L
Copper	15.00 mg/L
Iron	70.00 mg/L
Zinc	200 mg/L
Manganese	0.70 mg/L
Magnesium	450.00 mg/L
Potassium	2400.00 mg/L
Calcium	150.00 mg/L

comparable to the one of the molasses either 22.0 % of M.F (Table 1).

However, these musts are weakly provided in proteins and in phosphor in order of 0.24 to 1.05 % of M.F and 14.25 to 37.0 mg/100 ml of M.F, respectively.

By elsewhere,[4,14,15 and 16] appraised the needs in nutritious elements of the *Saccharomyces cerevisiae* by liter of medium of fermentation (Table 2).

The estimation of the needs of the yeast in these nutritious elements is based on the hypothesis that 100 kg of molasses having 50 kg in content of sugars gives about 25 kg of biomass. So, the musts of dates and molasses contain some quantities in sugars, potassium and calcium clearly superior to the needs of the yeast. By elsewhere, these musts are weakly provided in phosphor and proteins in order of 0.14 - 0.37 and 2.4 - 10.5 g/L, respectively.

These quantities cover 4.8 to 12.75 % some needs of the yeast in phosphor and 10 to 42 % some needs in proteins. In this sense, the addition of the ammoniac salts and phosphor like sources of nitrogen and phosphor is indispensable for the development of the yeasts during the fermentation. On the other hand, the microelements such as iron, zinc, manganese and molybdenum in minute quantities as components as activators of enzymes are also essential for the growth of the yeasts[17].

The results obtained demonstrated that the musts of dates contain some contents in magnesium, manganese and iron extensively sufficient in order to cover the needs of the bakery yeast, 450-700 mg/L, 0.7-1.4 and 22.0-58.6 mg/L, respectively. Finally, the biochemical analysis of musts of date show that these last could constitute a medium of fermentation of good quality with regard to this of molasses.

2/ Study of the Kinetic of Growth of the Different Strains of the Bakery Yeast: The compared study of curves of growth obtained with the different strains show that the latency time is relatively long with the SDN strain either 3 hours with regard to the other strains either 2 hours (Figure 1). Regarding to the rate of growth, this last is more elevated with the SDB strain either 0.47 h-1, respectively with regard to the SDN strain either 0.26 h-1. By elsewhere, the time of generation is more reduced with the SDB strain either 1 hour and 27 minutes with regard to the SDN strain, relatively more long either 2 hours and 36 minutes (Table 3).

The quantity of biomass and the neat yield obtained are more elevated with the SDB strain either 4.72 g/L of MS and 25.17 %, respectively with regard to those obtained with the SDN strain, 2.97 g/L of MS and 15.73 %. These results concords with those given

Table 3: Parameters of growth and yield of biomass of the strains of *saccharomyces cerevisiae*

Strains	ATCC1102	STB	SDN	SDB
Time of latency (hours and minutes)	2.0	2.0	3.0	2.0
Rate of growth in (h^{-1})	0.37	0.40	0.26	0.47
Time of generation in (hours and minutes)	1 h et 53 mn	1 h et 42 mn	2 h et 36 mn	1 h et27 mn
Residual sugars in % of M.F	0.10	0.062	1.12	0.125
Quantity of biomass in g/l de M.S	4.36	4.612	2.97	4.72
Neat yield in %	22.94	23.77	15.73	25.17

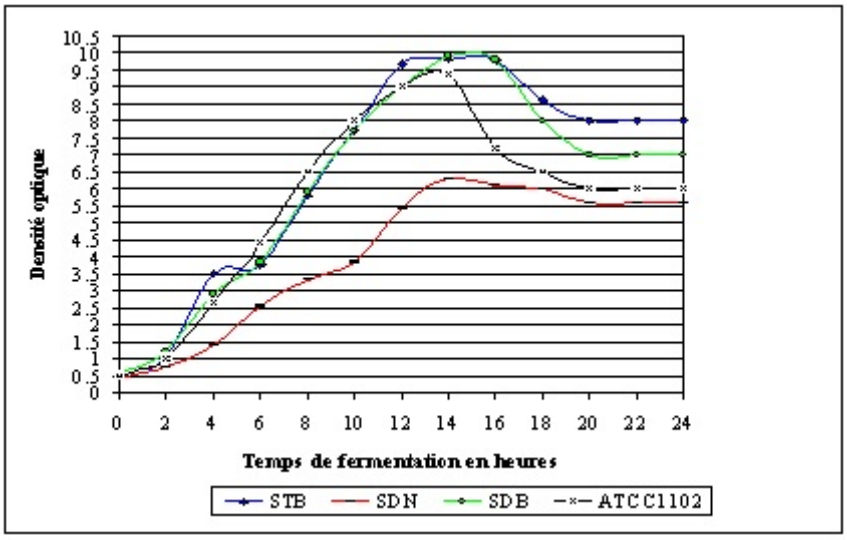

Fig. 1: Kinetic of growth of diffrent strains of *Sacharomyces cervisiae*

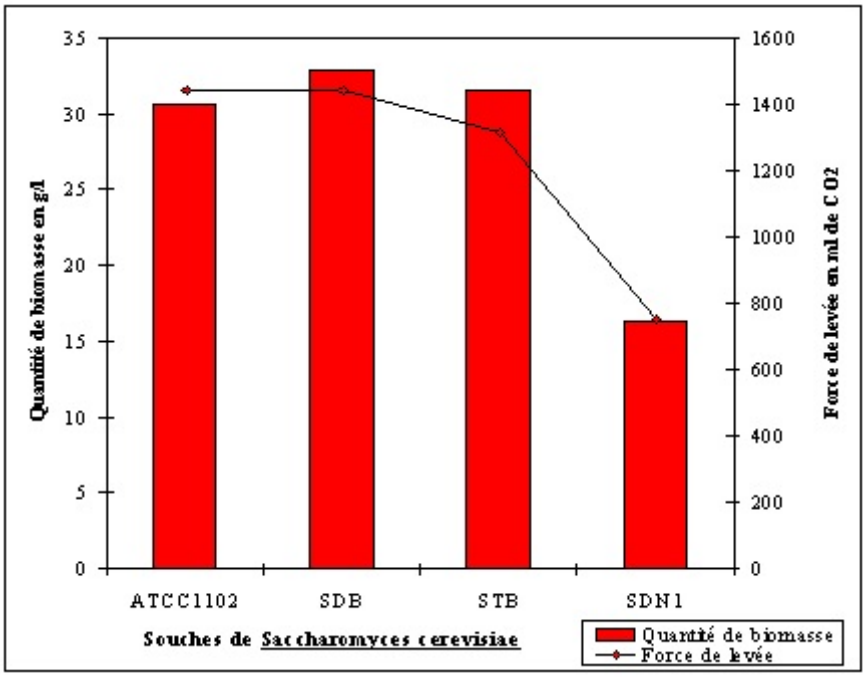

Fig. 2: Evolution of the quantity of biomass and the strength of levy following the diffrent strains

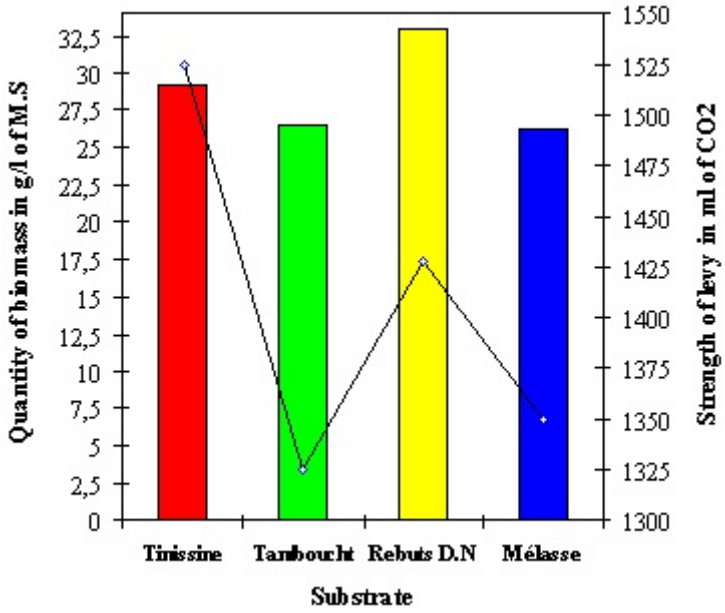

Fig. 3: Evolution of the quality of biomass and the strength of levy into function of the nature of substrate

by [18, 19 and 20] on molasses and dates either 3.0 - 4.6 g of M.S/L and 15.0 – 30.0 %, respectively.

Finally, the neat yield obtained with the different strains is weak, variable between 15.73 and 25.17 % with regard to the theoretical yield who is in the order of 45 % according to [21].

These weak neat yields obtained experimentally leaves to suppose that the sugar content in the medium is not completely consummate. Also, at the course of the fermentation, he produces not only the cellular mass and CO2, but some substances exo cellular who finds again in the medium. Nevertheless, the neat yield obtained is comparable to those obtained by [16, 20, 22, 23 and 24].

3/Production of the Bakery Yeast in Fed-Batch Fermentation: Yields of biomass varied considerably and were found to depend significantly on the strain of Saccharomyces cerevisiae used. So, the strains SDB and STB give higher quantities of biomass variable between 31.5 and 32.9 g/L, than the strain isolated from Deglet-Nour, SDN, either 16.3 g/L (Figure 2).

The similar results were reported by [18,19 and 25]. Regarding to the activity of the bakery yeast, we notes that the highest of strength of levy was obtained with the SDB strain either 1444.0 ml of CO2 and the weakest with the SDN strain either 752 ml of CO2.

By elsewhere, the quantity of biomass is more elevated with Offal's of Deglet-Nour medium, either 32.9 g of M.S/L with regard to the molasses medium, either 25.3 g of M.S/L (Figure 3).

The similar results were signaled by [6, 26]. However with Tantboucht, the obtained yield is weak, comparable to the one of the molasses, either 26.5 g of M.S/L.

This explains probably by the wealth of this last in copper and in zinc with regard to the other cultivars. In this sense, [16] signal that the concentration of 1.75 mg/L of copper in the medium of fermentation could reduce the cellular growth of the yeast to 50 %. Regarding the strength of levy, we note that the most elevated was obtained with Tinissine medium, either 1525.0 ml of CO2.

The weakest was obtained with Tantboucht medium, either 1325 ml of CO2. The lowest strength of levy obtained with Tantboucht medium is probably to the toxic effect of high level of copper. Baker yeast requires a minute amount of copper [4, 26, 27 and 28].

4/ Optimization of Bakery Yeast Production Cultivated in Fed-Batch Fermentation: According to[29], the supplementation of medium of fermentation with vitamins and amino acids facilitated efficient glucose uptake by *Saccharomyces cerevisiae*. To this effect, the comparative study of the different sources of nitrogen shows that the ammonium phosphate gives a yield in biomass more elevated variable between 45.37 and 45.47 g of MS/L with regard to the other sources of nitrogen (Figure 4).

The improvement in quantity of biomass obtained with this source of nitrogen is probably linked to bring of phosphor in substantial quantity indispensable to the development of the yeasts.

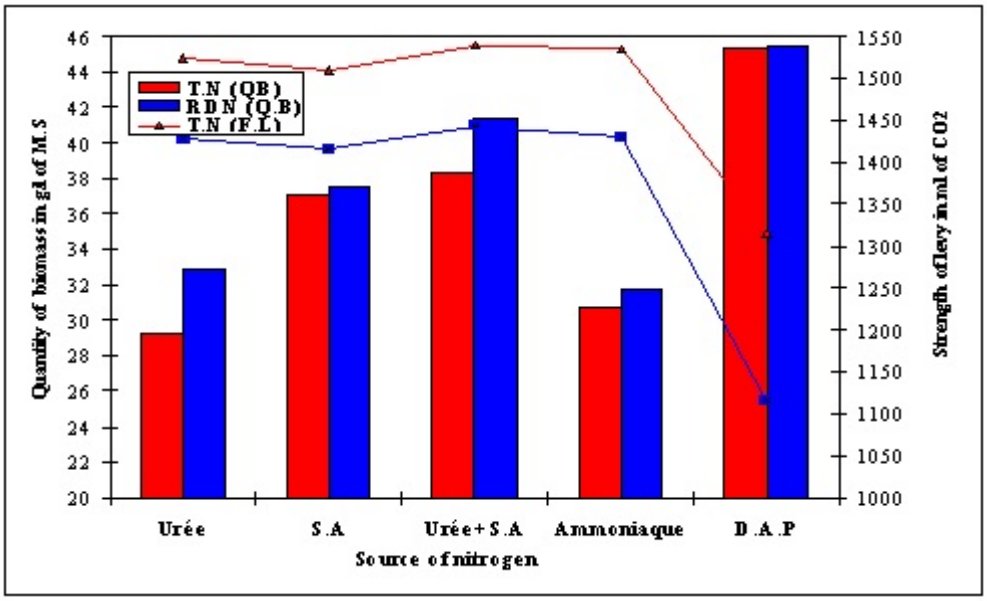

Fig. 4: Evolution of the quantity of biomass and the strength of levy into function of a source of nitrogen

Table 4: Quantity of biomass and strength of levy into function of a source of vitamin

Characters		Quantity of biomass in g/l of M.S		Strength of levy in ml of CO2	
Source of vitamin		Tinissine	R.D.N	Tinissine	R.D.N
Biotin	witness	37.60	40.75	1540.00	1444.00
	2 mg/l	38.40	40.50	1536.00	1441.00
	4 mg/l	38.70	41.80	1628.00	1627.00
	6 mg/l	39.30	41.40	1653.00	1644.00
	8 mg/l	38.90	42.20	1655.00	1639.00
Panthothenate of calcium	witness	37.60	40.78	1540.00	1444.00
	1 mg/l	37.40	40.10	1532.00	1443.00
	2 mg/l	37.90	40.60	1530.00	1423.00
	3 mg/l	38.10	40.60	1546.00	1449.00
	4 mg/l	38.90	41.00	1534.00	1446.00
Thiamin	witness	37.60	40.15	1540.00	1444.00
	0.2 mg/l	38.20	40.97	1552.00	1447.50
	0.4 mg/l	38.72	41.30	1549.00	1448.00
	0.6 mg/l	39.85	43.52	1556.00	1452.00
	0.8 mg/l	40.25	44.27	1570.00	1452.00

So, according to [16], it is necessary to the minus one part of P2O5 for three parts of NH2 consummate. The ammonium phosphate is interesting because the phosphor brought participle to the structure of the nucleic acids and some proteins of the constituent weightily important of the cell of yeast [12, 21]. However, this source of nitrogen is expensive with regard to the urea and to the ammonium sulfate. By elsewhere, no effect of the source of nitrogen on the strength of levy and this last varies between 1116 and 1540 ml of CO2.

Regarding to the source of vitamin, certain, such as, Biotin, Pantothenate of calcium and Thiamin are required for the growth of the bakery yeast as growth factors. Biotin participates in the synthesis of protein

and nucleic acid and in the formation of polysaccharides and fatty acids. Pantothenate of calcium influences the metabolic activity of yeast [28, 30]. This research show that the Biotin and the Panthothenate of calcium doesn't have any effect on the quantities of biomass produced (Table 4).

So, the quantities of biomass obtained with these vitamins vary between 37.4 - 39.3 g/L of M.S for Tinissine medium and between 40.1- 42.2 g/L of M.S for the offal's of Deglet-Nour medium. However, an improvement of the production of biomass was reported by [31] on the molasses medium containing sufficient quantity of Biotin. Nevertheless, with the Thiamin, an improvement of the quantity of biomass is noted and the optimal quantity requisite is 0.6 mg/L. Regarding to the strength of levy, the results show that beyond 4 mg/l of Biotin, he improvement of the strength of levy that passes of 1444 to 1639 ml of CO2 when offal's of Deglet-Nour medium was used and of 1540 to 1655 ml of CO2 when Tinissine medium was used. On the other hand, bring of Thiamine and Panthothenate of calcium to the medium of fermentation, doesn't have any effect on the strength of levy of the bakery yeast. The similar results were reported by [6, 27].

Conclusion: The results of this study indicate that the common dates and residues of dates can serve as a low-cost substrate for bakery yeast production by fermentation using Saccharomyces cerevisiae. So, the study of the kinetic of growth of four strains of *Saccharomyces cerevisiae* cultivated on offal's of Deglet-Nour, shows that the strain isolated from Degla-Beida (SDB) gives a good result to knowledge, a short time of generation, an elevated rate of growth and yield of biomass with regard to the other strains. By elsewhere, the results obtained in Fed-Batch fermentation shows that the date mediums and more particularly the Offal's of Deglet-Nour and Tinissine give an elevated yields in biomass with regard to the molasses medium. Nevertheless, the optimization of the parameters of production of the bakery yeast cultivated on date mediums in order to ameliorate the yields and the activity of the bakery yeast is desirable.

In this sense, the utilization of ammonium phosphate and ammonium sulfate + urea to 50 - 50 % ameliorates the yield of biomass. However, the ammonium phosphate is expensive with regard to the urea and to the ammonium sulfate. To this effect, we recommend the utilization of ammonium sulfate and of the urea to 50 - 50 % as source of nitrogen for the production of the bakery yeast. Finally, the obtained results show that it is not necessary to bring the vitamins during the course of the fermentation. This is probably linked to the wealth of the musts of dates in Biotin, Pantothenate of calcium and Thiamin, necessary to the developments of the yeasts.

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