

**Lactic acid production by a mixed culture of lactic bacteria based on low value Dates syrup**Djilali Bouhadi<sup>1</sup>, Bouziane Abouni<sup>2</sup>, Ahmed Hariri<sup>3</sup>, Kada Ibri<sup>4</sup>, Nawel Ouis<sup>5</sup>, Fatima Sahnouni<sup>6</sup><sup>1</sup>Bioconversion Laboratory, Microbiology engineering and health Safety,

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**Abstract:** The common varieties of dates represent about 30% of national production. They are generally reserved for cattle feeding and sometimes thrown into the wild. Among these varieties, the Ghars that is very rich in carbohydrates (glucose, fructose and sucrose) and contains practically most of minerals (Ca, Fe, K, Na, etc...). The use of fermentation technology is necessary to save the genetic heritage and enhances by-product of dates in various bio products. Lactic acid is a natural organic acid. The fermentation substrates most commonly used are glucose and fructose. The lactic acid has a major importance in food and pharmaceutical industries, it is used also in drink industries, medicines etc. Algeria continues till today to import this organic acid.

The objective of this study is the use of dates syrup as a substrate for the production of lactic acid, this study allowed us to have interesting results. Mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, grown in batch fermentation. Two culture mediums were tested, the un-enriched one and the one enriched with Tween80 (1g/l), MgSO<sub>4</sub> (1g/l), MnSO<sub>4</sub> (1.5g/l), from the results obtained by fermentation trials, the enriched medium seems best suited with a yield of lactic acid 49g/l, where comes from its economical and commercial interest.

**Key words:** Lactic acid, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, date syrup, fermentation.

## INTRODUCTION

Phoenicicole heritage spreads on more than 85.000ha and reckons with more than 13 million palm trees, producing an average of 400,000 tons and allowing Algeria to the 5<sup>th</sup> place among the world producers of dates <sup>[1]</sup>.

The phonicicole Algerian sector provides date varieties of high quality and excellent commercial value. Other varieties called common are less appreciated, less commercialized and less competitive, in return they are very rich in nutriment, sugars such as: glucose, fructose and sucrose, vitamins and minerals <sup>[2,3]</sup>. Subsequently, they provide a very high energy. The use of transformation technology shows that it's more necessary to use low-value dates for livestock feed <sup>[6]</sup>.

Adding also that the dates have already been the subject of many tests in other countries. They are exploited as fermentation substrate for various metabolites production such as citric acid, ethanol and even biomass production (yeast bread) <sup>[4,5]</sup>.

Lactic acid or its derivatives are indeed widely used by the pharmaceutical industry, food processing and chemical industries for the synthesis of varnishes and plastics <sup>[7]</sup>. The biotechnology evolution and the integration of microbiology and biochemistry enabled to master fermentation techniques and processes by involving an appropriate substance for lactic acid production as molasses, carob and glucose <sup>[8]</sup>.

The objective of this work consists to use a second quality date's syrup as a fermentation culture medium in the purpose of producing lactic acid by a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in different experimental conditions.

## MATERIALS AND METHODS

### 1/Materials

**1.1/ Vegetable Material:** The vegetable material used in current experiments was a half soft variety, known as Ghars badly exploited, cultivated in the area of Adrar (South-western of Algeria). The choice of this variety is justified by its availability and important nutritive value, especially the one of reducing sugars.

### 1.2/Biologic Material:

The biologic material used is cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, derived from Orolait of Mascara (Algeria). The mixed culture is homofermentative (homolactic) which means that more than 90% of metabolites are produced by the lactic fermentation. The interest of mixed cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* is to start the phenomenon of symbiosis that is characterized by metabolic interactions between the two strains. This positive interaction demonstrated by comparing the lactic acid production in pure and mixed cultures of two species. The amount of lactic acid produced by the mixed culture is greater than the sum of acids produced by each of the pure cultures <sup>[16]</sup>.

### 2/ Experimental Protocol

**2.2/ Preparation of date syrup:** The dates are washed themselves, destoned and grounded. Two and 1/2 liters of hot water at 80-85°C were added to 1kg of date, homogenized and through a cloth. The syrup obtained was centrifuged at 15.000 rpm for 10 minutes to separate the cellulose debris. The collected supernatant was used as culture medium. The syrup is sterilized during 20 minutes at 120°C.

### Batch Fermentation:

Batch cultures were performed in 250 ml Erlenmeyer with a working volume of 100 ml,

inoculated in the presence of MRS medium (*Lactobacillus bulgaricus*) and M17 medium (*Streptococcus thermophilus*) and incubated for 42h for 24h, similar cultures were equally conducted in a reactor having a capacity of five liters provided with all the accessories. The temperature of fermentation is maintained constant to 42°C and agitation is of 300 turns per minute. pH fixed at 6.1 because the development of mixed culture needs a pH between 5.8 and 6.5).

The biomass evolution, sugar consumption and lactic acid production are followed at regular time intervals.

### 3/ Analytical Methods

**3.1/ Biochemical Analysis:** The content in water is determined by drying 10 ml of syrup at 105°C during 18 hour <sup>[9]</sup>. The content in ashes is determined by incineration one gram of syrup at a temperature of 600°C during 3 hours <sup>[9]</sup>. The reducing sugars, the sucrose and the total sugars were determined by the method of Bertrand, reported by <sup>[10]</sup>. The total nitrogen is determined by the method of Kjeldah <sup>[10]</sup>. The mineral salts are determined according to the methods advocated by <sup>[11]</sup>.

**3.2/ Kinetic of Growth:** We take off every two hours until 28 hours 10 ml of medium of fermentation. We make a reading in a spectrophotometer to a length of wave 620 nm.

**3.3/ Lactic acid:** Concentration of the lactic acid was determined by acidity titration with NaOH <sup>[10]</sup>.

**Residual sugar:** The content of residual sugar was determined by the method of Bertrand, reported by <sup>[9]</sup>.

### 3.4/ Data processing

The calculation of the kinetic parameters of fermentations has been performed using KALEIDAGRAPH software, is a specific program for the graphs, the histograms and of statistical calculation.

## RESULTS AND DISCUSSIONS

### 1/ Biochemical Composition of Syrup of Dates

The date syrup which has been the subject of our work has a high water content 88.69g/l (Table1), we agree that a product with a high water content facilitates lactic acid bacteria proliferation and helps for a better substrate -enzyme contact since free water is the nutriment carrier, knowing that *Lactobacillus bulgaricus* needs a minimum water activity of 0.992 to grow while *Streptococcus thermophilus* needs a minimum of 0.983.

Date's syrup will be very rich of total sugars 56.8g/l among them a rate of 4.16g/l for sucrose. [12] Indicates a grade of 84g/l of total sugars in the soluble extract of date's flour. These differences are explained by climatic variations. Reducing sugars are a carbon source that can satisfy the ferments requirements.

The protein fraction is considerable; therefore it can serve as a nitrogen source. An ash content of 0.9g/l indicates its richness of minerals including potassium (280), sodium (180mg/100ml of MF), phosphorus (28 mg/100 ml of MF) and calcium (146 mg/100 ml of MF) and a very small amount of magnesium (0.11 mg/100 ml of MF) and manganese (0.19 mg/100 ml of MF).

The date's syrup has an acidic pH 5.9 close to neutrality which indicates its good nutritional quality (no bacterium contamination) suitable for the proper development of lactic acid bacteria. Similarly <sup>[15]</sup> stated that the majority of common varieties (dates of average quality) have pH ranging between 5.3 and 6.3.

The analysis applied on the date syrup shows that it is poor, with minerals such as Magnesium, Manganese and fatty-acids, so the addition of these elements (growth factors) is necessary to the syrup in order to import this quantity of lactic acid.

Finally, the biochemical analysis of date's syrup show that it can constitute a fermentation medium of good quality.

**Table:** Biochemical Composition of Syrup of Dates

Constituents	date syrup
Content in water in %	88.69
pH	5.9
Total sugars in % of M.F	56.8
Reducing sugars in % of M.F	26.8
Sucrose in % of M.F	4.16
Proteins in % of M.F	1.02
Ashes in % of M.F	0.9
Potassium in mg/100ml of M.F	280
Phosphor in mg/100ml of M.F	28
Sodium mg/100ml of M.F	180
Calcium in mg/100ml of M.F	146
Zinc in mg/100ml of M.F	0.19
Copper in mg/100ml of M.F	0.17

Magnesium in mg/100ml of M.F	0.11
Manganese in mg/100ml of M.F	0.19

## 2/ Results of the fermentation kinetics

The production curves of lactic acid in enriched and un-enriched mediums looked the same, the production in the un-enriched begins with an initial rate of 0.12g/l to achieve 26g/l of lactate in 28h of fermentation (figure1). In parallel, in the medium enriched with Tween80, MgSO<sub>4</sub> and MnSO<sub>4</sub>, the lactic acid rate evolves gradually to achieve the end of fermentation 49g/l.

Meanwhile, the biomass evolution in the enriched medium starts with an initial concentration (OD=0.04) and after 28h of fermentation it reaches a maximum value (OD=2.40) (figure2). In the case of non-enriched one we observe a low initial concentration of biomass and in the end of fermentation optical density reaches a maximum value 1.15.

However, decreasing of sugar rates is very faster in the medium supplemented with MgSO<sub>4</sub>, MnSO<sub>4</sub> and Tween80. Where the mixed culture consumes about half quantity of initial total sugars, during 28h of fermentation from an initial quantity of 56.8g/l of sugars, it remains 31.3g/l which implies an amount of 25.5g/l (figure3). In parallel 11.7g/l as the quantity of sugar consumed in non-enriched medium.

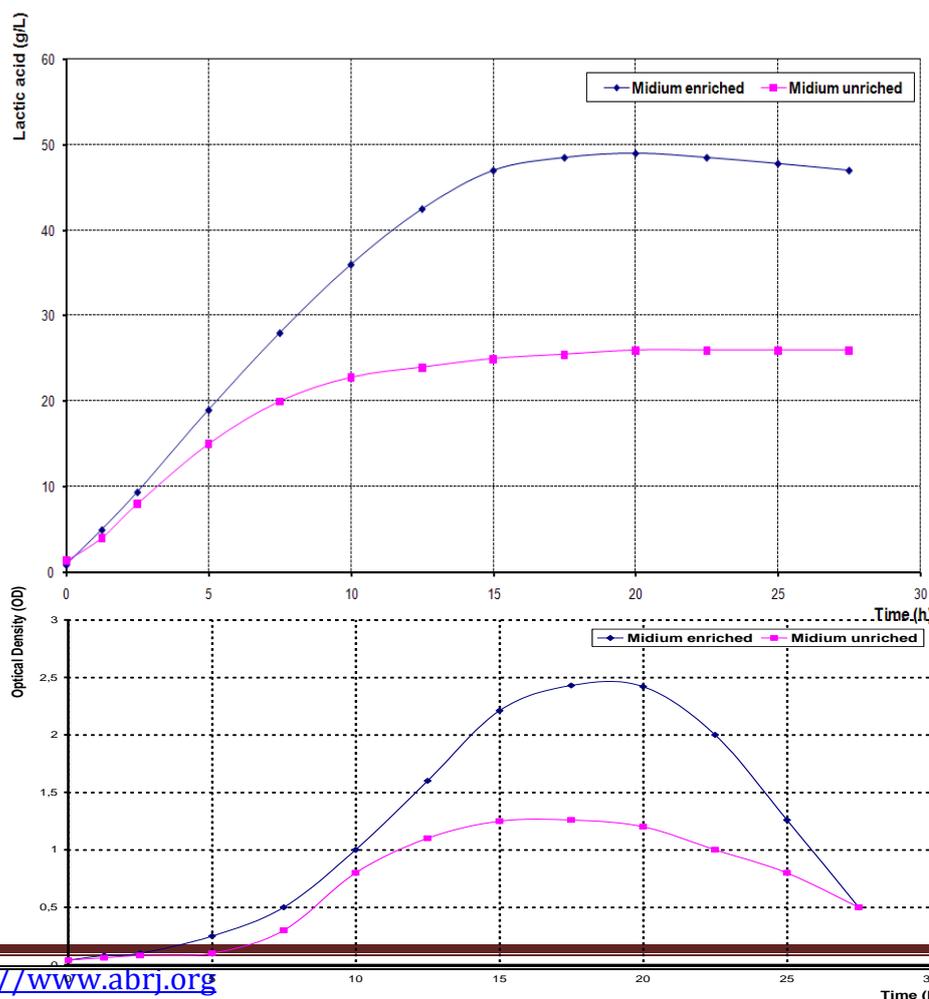
Comparing the results for both fermentations indicated that the addition of growth factors in culture medium has a positive and beneficial effect on fermentation, since the growth rate and lactic acid production increase after the enrichment of date syrup.

According to <sup>[14]</sup>, the addition of Tween80 allows better cells excretion of lactate by creating pores in the membrane and plays the role of surfactant which makes a good contact between seed and nutrients, and for more it is considered as a source of carbon and energy for electrons.

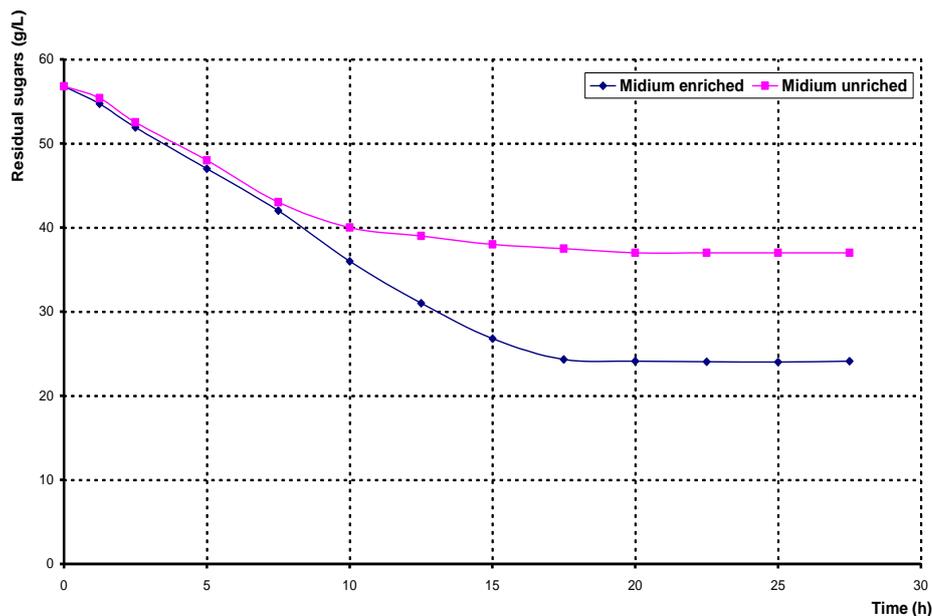
According to <sup>[15]</sup>, the presence of manganese in the medium is specific and can not be replaced by other minerals, because the Mg<sup>++</sup> enters in the structure and functioning of enzymes.

By the same author, the addition of Manganese stimulates the proliferation of *Streptococcus thermophilus* and it is essential also for *Lactobacillus bulgaricus*, as Mn<sup>++</sup> is the activator of various metabolic reactions, it participates in the transfer of lactate across the cytoplasmic membrane to be released in the medium.

From these results, the enriched medium seems the best for its interesting results. Indeed, a production of 49g/l of lactic acid obtained with medium based on date syrup enriched with Tween80, MgSO<sub>4</sub> and MnSO<sub>4</sub> is higher comparing to the results found by <sup>[18]</sup>.



**Figure1:**  
Evolution of  
lactic acid rate  
produced by  
both  
fermentations.

**Figure2:** Evolution of optical density for both fermentations during time.

residual sugars rate for both fermentations during time.

**Figure 3:**  
Evolution of

### 3/ the kinetic parameters of fermentation

Changes over time of specific growth rates ( $\mu$ ), lactate production ( $Q_L$ ) and substrate consumption ( $Q_S$ ) are presented in three figures 4, 5 and 6.

In un-enriched medium the specific growth speed ( $\mu$ ), after 3 hours of fermentation is of  $0.38h^{-1}$  (figure 4). For the culture enriched with Tween80,  $MgSO_4$   $MnSO_4$  the specific growth rate begins with a top speed of  $1h^{-1}$  then decreases with time to the point of cancellation, which corresponds to 18 hours of culture time.

Basing on these results, we can say that the earlier addition of manganese, magnesium and Tween80 in fermentation helps in improving culture, enhancing and promoting cell proliferation and metabolic reactions. The decreasing in specific growth rates in the two fermentations can be explained by the disappearance of the culture medium, one or more essential elements to the maintenance and cell growth, it can be due also to the realizing of culture medium by using substances that have been fatal for the growth; this causes despite the abundance of sugar (carbon source) cell lyses and death of cells.

Specific speed of sugar consumption is very important  $16g/g.h$  in un-enriched fermentation, whereas it is  $7g/g.h$  for fermentation supplemented with Tween80,  $MnSO_4$  and  $MgSO_4$  (figure5). This is logical since the maximum amount of biomass obtained for the first fermentation (unenriched) does not exceed  $1g/l$ , and to calculate this rate we divide the changes of consumed sugar by biomass variation.

After a time of fermentation, the specific rates of sugars consumption for both fermentations start to decline and to be cancelled after 18 hours of fermentation, which correspond to cells death described above.

Specific speeds of lactic acid production in the two fermentations give identical and maximum values of  $2g/g.h$  (figure6), they are superimposed and decline together to be cancelled after 18 hours of fermentation.

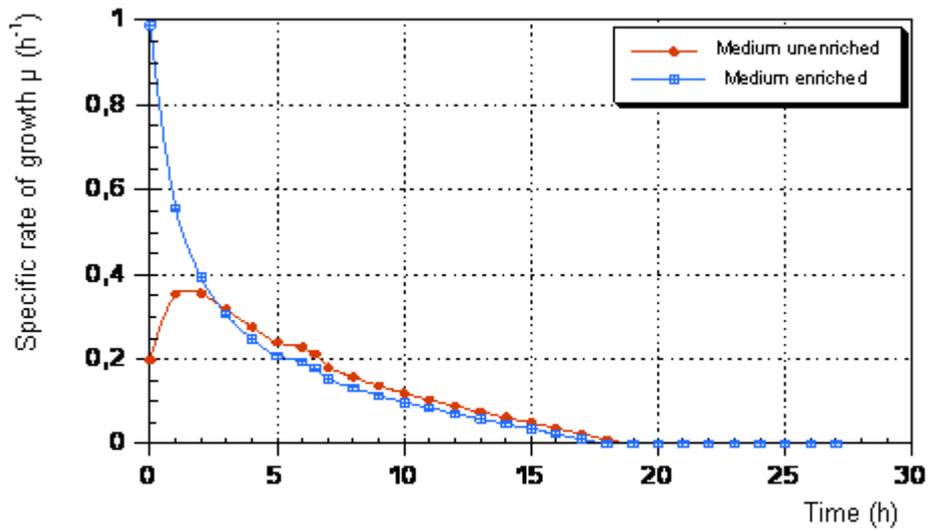
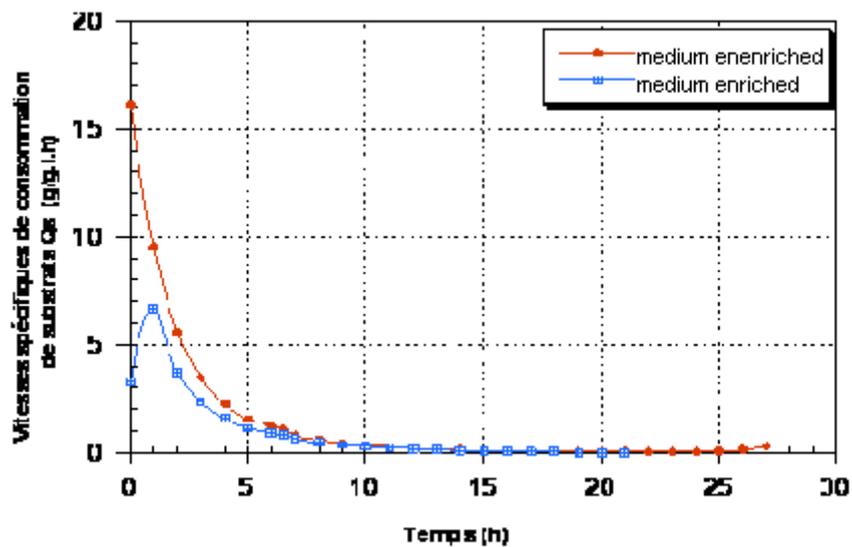
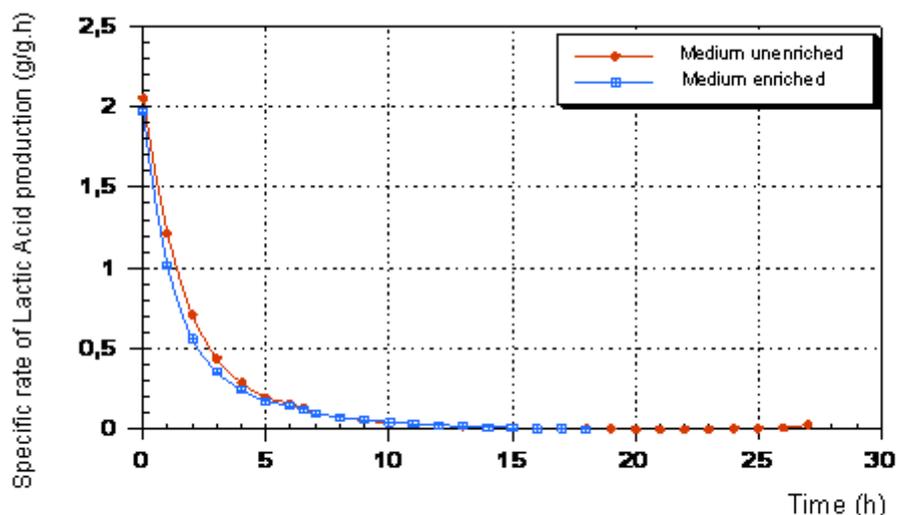


Figure 4: Evolution of specific growth rates ( $\mu$ ) for both fermentations during time .



**Figure 5:** Evolution of specific rates of sugar consumption ( $Q_s$ ) for both fermentations during time.**Figure 6:** Evolution of specific speeds of lactic acid production ( $Q_L$ ) for both fermentations during time.

### Conclusion

The bioconversion of agricultural by-products mainly the ones rich in fermentable sugars has an economic and strategic interest.

By its biochemical composition, the date's syrup is very rich in carbohydrates 56.8g/l. which make it a substrate of choice for the development of high value substances.

The kinetics of growth under our experimental conditions showed that the date's syrup is a growing medium that induces the biosynthesis and production of lactic acid in two lactic mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The results obtained from the fermentation trials show that the mixed culture can produce 26g/l of lactic acid in medium based on date's syrup. This medium requires an addition of Magnesium, Manganese and Tween80 to allow an adequate growth and maximum production of lactic acid 49g/l.

At the end of this study, it appears that the valuation of date's syrup by biological means (fermentation) for the production of lactic acid has certainly huge advantages.

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