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ECONOMICAL CITRIC ACID PRODUCTION USING COMMON DATES FROM ALGERIA

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Abstract

The current research concerns the valorization of two varieties of common dates (dried variety Mech-Degla and the wild variety) in sight a better citric acid bioconversion. In this context, various media (syrups and suspension at base of the pieces of dates) were developed. These media were Fermented by Aspergillus Niger by applying two modes of culture (submerged and on the surface). Furthermore, a comparison between certain parameters of fermentation (growth rate, citric acid yield,..) were given. Then, the choice of the optimal medium to produce critique acid is subjected to statistical treatment (ANOVA) and NEWMAN KEULS test at ρ=5%. The results show that, Mech-Degla variety is more interesting than the wild variety. The media prepared with this variety is found to be characterized by significant levels of sugar (4%) allowing a better production in citric acid (7.5±0.04g dry weight/100ml/14 days, n=3). According to the variance analysis with two criteria of classification reveals a non-significant difference at 99% probability (F=0) for the two factors (nature of the medium and type of culture). Therefore, this study would continue using multicriteria optimization. The bioconversion of dates in citric acid by A.niger yields a better exploitation of the local resources.

Keywords: *common dates, Aspergillus niger, bioconversion, citric acid*

1. Introduction

Algeria is a producer of dates with an annual production of 261 000 tonnes for normal companion. Almost half of this amount (53%) consiste the varieties of common dates (dried dates low market value). Despite its importance, more than 14,000 tons of these dates are destined for animal feed (Ouldelhadj et al. 2012).

Common dates have an undeniable socio-economic and nutritional importance. The richnessof these date sugars offer them the opportunity to be recycled in various products (syrup, vinegar, alcohol, yeast ....) (Acourene and Tama, 2001). Despite the alimentary importance and interest to it, use and date processing technology in Algeria remain limited (packaging and pulp production).

Several research studies have shown the importance of the use of the variety of dried dates *Mech-Degla* in the preparation of various food products such as yoghurt and tablets made from date powder (Benamara et al. 2008, Benahmed Djilali et al. 2011).

In addition, the date palm is a substrate of choice for the production of high value-added substances. In particular, organic citric acid was produced by*Aspergillus Niger* responsible for the degradation and synthesis of compounds that can be used in various fields: biotechnology, agriculture, the food industry and / or environment (Antonio, 2014).

The production of natural acid extracted from the fruit cannot cover this growing demand.

Said mold, is the microorganism of choice in the industrial scale due to its ease of cultivation, genetic stability, its high yields, its inexpensive use of capacity and low production of undesirable metabolites (Zergat, 1996; Siboukeur et al. 2001).

Moreover, the success of the culture of a mold is closely related to the quality of the substrate used. Thus, it is necessary to search for other growing media to massively produce organic products at low prices, but also, the minerals that will exhaust in this environment with a good mastery of techniques and culture conditions.

In this context, two varieties of dates (*Mech-Degla*) and non-edible wild dates were subjected to metabolism in the presence of a wild strain *Aspergillus Niger* inappropriate for the purpose of analyzing the possibility of formulating the bio citric acid in two cropping patterns on the superficiel and submerged.

2. Materials and methods

2.1.Plantmaterial

Two varieties of dates were used during this study namely the dry variety *Mech-Degla* which belongs to the category of common dates bought at the local market in the town of Reghaia (Algiers) during the spring of 2014; it is a native of Biskra. This variety is recognized by its abundance, richness in sugar (sucrose) and its low water content (less than 20% moisture) (Espiard, 2002; Benahmed Djilali, 2012).
The second variety which is an inedible date is grown in northern Algeria; it comes from the city of Tizi-Ouzou. Both varieties were stored in a refrigerator at 4 ° C until processing and / or analyze, in order to slow respiration, chemical and physiological changes (Maskan, 2002).

2.2. Biological material

The species *Aspergillus niger* used for fermentation was provided to us by the microbiology laboratory of the CHU hospital of the city of Tizi-Ouzou. The spores of this strain were grown on Sabouraud medium with chloramphenicol at 22 ° C for 7days. The spore count was performed using the Malassez-cell.

The initial concentration of *A.niger* spores used to inoculate all circles is 1.5 10^7 spores / ml. The inoculation was carried out with a ratio (10/100) (v / v) inoculum / fermentation medium. The bioconversion of organic citric acid dates was conducted using two fermentation modes (surface and submerged) in order to seek maximum yield of citric acid.

2.3. Fermentation media

The fermentation was carried out in syrups and cut dates suspensions prepared from the two varieties of studied dates. The syrup was obtained by soaking a mass of dough of dates in water with a ratio (1/3) (g / g) of dates / water paste. The mixture is heated to 75 ° C in a water bath for 3 h with manual agitation. At the end filtering is performed in order to extract as much sugar. As regards the suspension based on pieces of dates was prepared in accordance with the same ratio (1/3) (g / g) pieces of dates / water.
All prepared media were sterilized and then stored at 04 ° C prior to fermentation with the aim of avoiding the bioconversion of fermentable sugars.

2.4. Methods

2.4.1. Settings mycelial growth

Mycelial growth was estimated by the method of spore count on Malassez cell using an optical microscope (Notic SFC- 18 series).The microstructure of dried mycelial biomasse was studied by environemental Scanning Electron Microscope SEM (PHILIPS ESEM XL.30; Heindoven, Netherlands). Samples were prepared without metallization according to the protocol recommended for particle size of 200µm. Two parameters of mycelial growth were determined during the exponential phase for various fermentation media. This growth rate (µmax) and the generation time (tg).

Monitoring the pH and the change in reducing sugar content were also determined.
After 14 days of fermentation, one proceeds to the crystallization of citric acid according to the method described by (Gourgard and Boar, 1995), and the yield was calculated with respect to reducing sugar levels according to the following formula:

$$Citric\ acid\ yields\ (\%) = dried\ mass\ of\ citric\ acid/mass\ of\ consomed\ reducteurs\ sugrs \times 100 \ldots (1)$$

2.4.2.IR analysis of citric acid

In order to verify the purity of our citric acid produced by fermentation, IR spectrometry (BRUKE ALPHA, China) was used to analyze the functional groupings of three types of citric acid (citric A. marketed food, citric A derived from chemical synthesis brand (BIOCHEM, Chemopharma) and obtained citric A) was performed in the laboratory of Chemistry of the University of Mouloud Mammeri Tizi-Ouzou.

2.4.3.SEM analysis

The microstructure of powders of three citric acids (B: citric acid chemical; D: citric acid used in food and F: biocitric acid) was studied by environemental Scanning Electron Microscope SEM (PHILIPS ESEM XL.30; Heindoven, Netherlands). Samples were prepared without metallization according to the protocol recommended.

2.4.4.Statistical study

Results in yields of citric acid obtained by using the various fermentation media were subjected to an analysis of variance of two factors: factor 1 (cultivation method on the surface or submerged) and factor 2 (used syrup substrate capable or suspension of chopped dates).
The statistical model used for this analysis is done with the Statistica BOX software version 6.4 with the coefficients of K.PEARSON. Medium were subjected to the homogeneous test groups according to the method of Newman Keuls analysis by ANOVA.

3. Results and Discussion

This study shows that the *Mech-Degla* variety is a favorable environment for the mycelial growth and the production of citric acid in the two cultivation methods studied. In comparison with the second wild variety, the bioconversion of citric acid did not occur. This is related to the biochemical composition of dates (low reducing sugar content, rich in tannin compounds responsible for the inhibition of growth, ...). Indeed, the sugar concentration in dates depends on the time of harvest, cultivation and environment.

Regarding mycelial growth, both varieties promote good mycelial output in the case of the culture surface using different media (syrups or suspensions dates) thereby forming a thick layer of 5 to 8 mm in average from 8th day of fermentation. Average yields ranging from 13-15 g FM / 100ml in the case of metabolism syrups dates (wild and *Mech-Degla*), respectively. By analyzing the various measured parameters (growth rate and yield of citric acid) (Fig 1 and 2), we found that the production of citric acid by *Aspergillus niger* involves two major consecutive steges:

1- The first stage lasts eight days and is characterized by mycelial growth linked to a high intake of sugars. However, the secretion of acid is very small.
2- The second stage last six days shows an accumulation of citrate. Where by growth is inhibited. This inhibition growth was indicated by the flocculation phenomenon, which is due to the lowering of pH (near 2.8) in the different environments studied in the two cultivation methods adopted.

Based on these results, we can infer that the metabolism of sugars by *A.niger* citrate follows the type of reaction (II) according to the classification of Gaden.

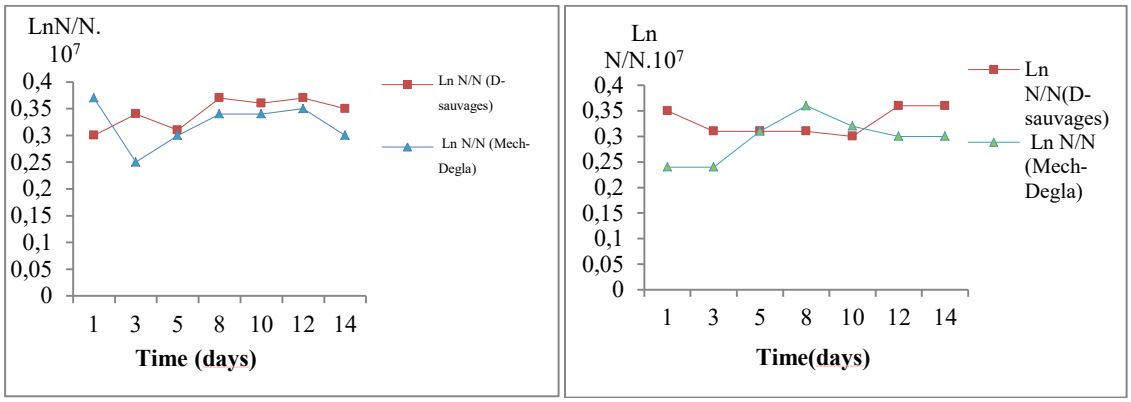


Fig 1: Semi-logarithmic growth curve of *A. niger* in syrop dates.
a) : superficiel culture ; b) : Submerged culture

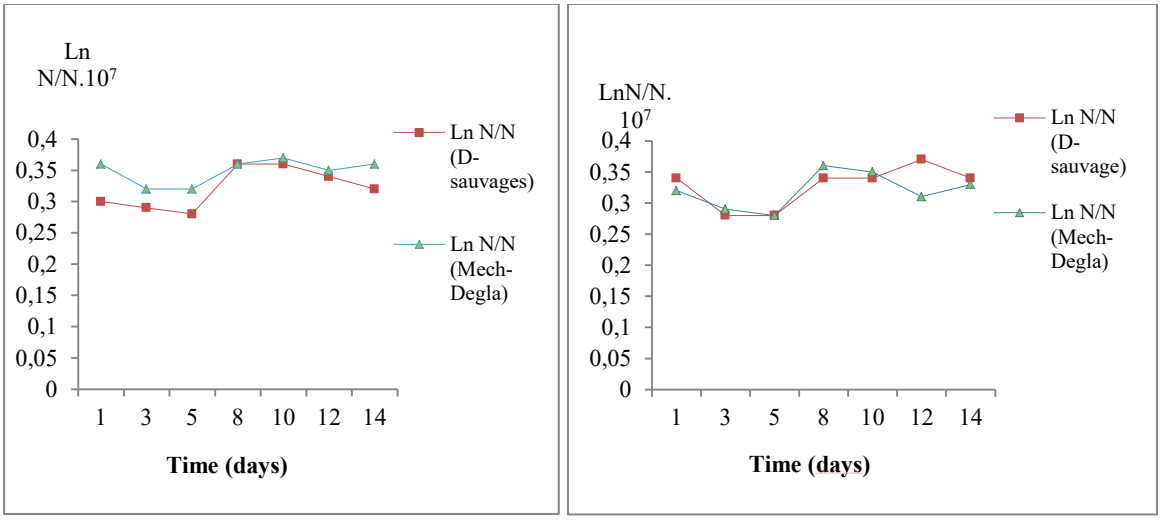


Fig 2: Semi-logarithmic growth curve of A. niger in cut dates suspensions
a): Superficiel culture; b) : Submerged culture

Comparing these results with other research, it is important to note that the success of citric fermentation is related not only to the acidity of the medium but also the sugar content. Our results are consistent with those reported by Saddek and Fouzi (1993), which showed that the best yields are obtained at pH 2.5.

By cons, other researchers to focus more on the sugar content considered to be a limiting factor responsible for obtaining good yields.

Indeed, Simon and Miller (1970) indicate that the concentration of sugars in the wort should be between 12 and 15%. Similarly, Jernejc and Legisa (2004) suggested a sugar content of about 14% (Xu et al. 1989) noted the absence of citrate to media containing less than 2.5% sugar. The latter, called for a rate sugar ranging from 10 to 14%. Table 1 summarizes the growth rates and yields obtained in citric acid fermentation after 14 days of culture in the two modes using the same variety *Mech-Degla* .

Table 1: Growth rate and yield of citric acid (cas *Mech-Degla*, n = 3)

Type of culture ***Substrate***		**μ (h^{-1})**	**Initial reducing sugars (g)**	**Citric acid mass (g)**	**Y(%) of citric acid in 100 ml of substrate**
Superficiel Culture	Syrop	0.93	4	0.2	5
	Cut Dates Suspension	0.08	6	0.2	0.33
Submerged Culture	Syrop	0.61	4	0.3	7.5
	Cut Dates Suspension	0.37	6	0.3	5

The production recorded in this study is small compared with those reported in the literature. This difference might indeed be due to the low concentration of spores inoculated and sugars. Nevertheless, the results argue that the *Mech-Degla* variety seems more interesting than the wild variety as it allows better citrate production that deserves further optimization study.

Analysis of variance with two classification criteria: 1) type of culture (submerged or superficiel) and 2): substrate type (syrup or cut dates suspension) revealed a non-significant difference between the citric acid yields with a probability of p = 0.19 and F = 10.917 (Table 2) using the same variety of *Mech-Degla* dates. This allowed us to infer that the method of cultivation and the substrate nature does not affect performance in citric acid. By cons, there are other factors (sugar content, source of oxygen, minerals ...) which can influence citric fermentation (Elogaidi 1987; Zergat, 1996).

Table 2 : Statistical results

Facteur 1	**Surface Culture**	**Submerged Culture**	**Surface Culture**	**Submeged Culture**
Facteur 2	Syrop	Syrop	Cut dates suspension	Cut Dates suspension
Variance	12,852	12,852	12,852	12,852
moyenne	2,665	6,25	2,665	6,25

Facteur 1 : *type of culture* ; Facteur 2 : *Nature of substrat*

The IR results show that citric acid used in food (Fig 3.D) shows no peak. However, citric acid used in chemistry (Fig 3.B) and that obtained from the fermentation (Fig 3F) show the appearance of the same absorption bands (1050 to 1085 and 1730 to 1750 cm $^{-1}$) attributed primary alcohols and aliphatic esters, respectively.

A peak at 2960cm-alkanes attribute only appears in the citric acid used in chemistry. It was noted that the biological process improves the quality of the acid obtained by the appearance of new absorption bands (750-800, 1500 and 3010-3040cm^{-1}) allocated to three new functional groups corresponding to compounds respectively, aromatic, nitro-aromatic moiety and alkenes and which are absent in the citric acid from the chemical process.

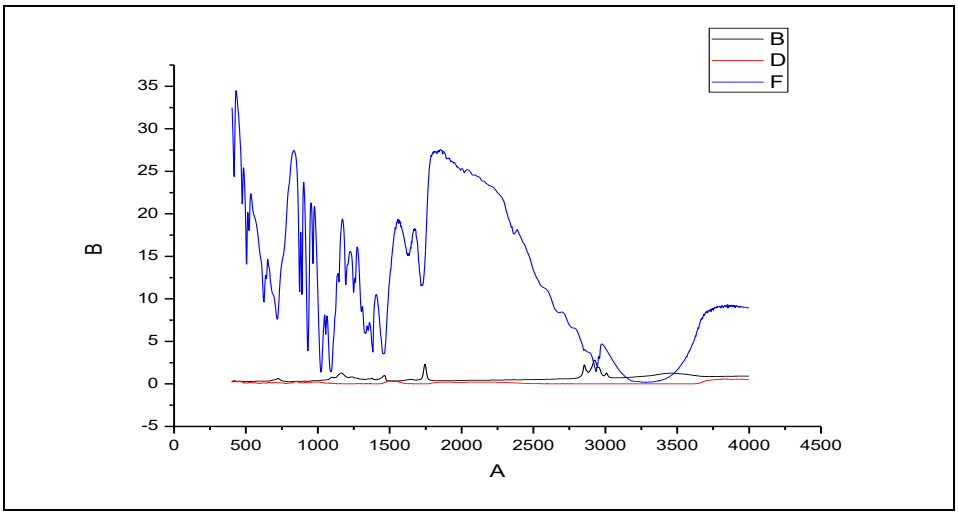


Fig 3 : IR Spectre of citric acid anlysed

B : citric acid chemical; D :citric acid used in food and F : biocitric acid

This difference in the composition also affirms the differente microscopic structures of the three analyzed acids. Indeed, citric organic acid (Fig 4 a) has a structure in the form of sheets stacked forming the rods. Whereas, the other two acids are characterized by similar structures in the form of complex agglomerates.

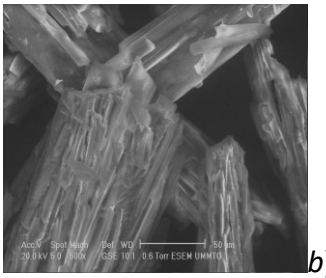
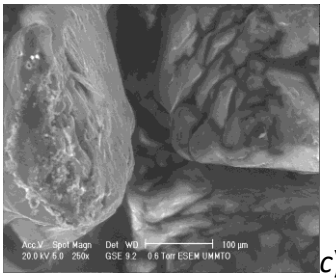
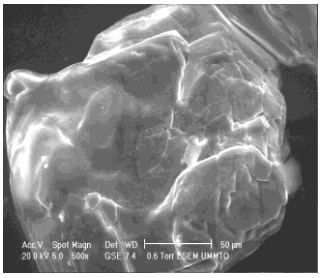
a)  *b*)  *c*) 

Fig 4: microstructures of three citric acids studied.
a):Bio-citric acid ;b):Chemical citric acid; c):citric acid used in food.

4. Conclusion

It would be interesting to conduct research on the optimization of fermentation conditions such that agitation and oxygen source. Aeration particularly because it plays a crucial role in the development of microorganisms.

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