

Valorization of Date Waste: Comparative Study between the Dry Variety of Dates *Mech-Degla* and the Must of Fermentation

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Abstract. The fruit of the date palm (*Phoenix dactylifera*) is one of the most abundant fruits in the Middle East and North Africa. Waste such as date pits represents an average of 10% of the date fruits. The aim of the study is to valorize dates of low market value *Mech-Degla*. The approach consists first, to better know this raw material, and therefore to analyze it, which will make it possible to detect the possible qualities of these dates. The physico-chemical characterization of date waste reveals the following results: The pulp of the date has a weight percentage of 82.79% in the whole fruit, a humidity of 14.4 ± 0.35 , a slightly acidic pH of 5.62, acidity at 0.24% (fresh material), a reducing sugar content of 24.23% (FM) and 2% (FM) ashes. The fermentation must prepared from these dates is characterized by a decrease in pH to 3.4, a reduction in the content of reducing sugars (4.88%), and ashes (0.3%). Acidity increased by 1.1%, the biomass is 3g/l (dry matter) at the end of the fermentation. The bacterial strains isolated from the must are represented by Y (*Saccharomyces*), SNC (*Penicillium*), S (*Leuconostoc*), U (*Pediococcus*), X (*Leuconostoc*), Z (*Pediococcus*). Use of waste the date in the production processes is likely.

Keywords: Date waste, Fermentation, *Mech-Degla*, Must, Valorization.

1. Introduction

Phoenix dactylifera commonly known as date palm is a monocotyledonous plant of the family Areaceae, important in the regions of North Africa and South-West Asia [1], [2] and [3].

The date is the edible fruit of the date palm (*Phoenix dactylifera*). Date is one of the oldest known fruit crops at least 5000 years [4]. Dates are rich in certain nutrients and provide a good source of fast energy, because of their high carbohydrate content (70-80%). In addition the fruits contain date fat (0.2-0.5%), protein (2.3-5.6%), dietary fiber (from 6.4 to 11.5%), mineral salts (0.10 to 916 mg / 100 g dry weight), some vitamins (C, B1, B2, B3 and A) with very little or no starch [5].

However, thanks to biotechnological processes, it would be possible to put on the market a new generation of products whose socio-economic impact is considerable. Date wastes crystallize up to 65% fermentable sugars and therefore represent a substrate of choice for the production of many substances with high added value; among other things ethyl alcohol strategic energy substance and base of many industries [6].

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The substitution of molasses with date must be of great economic importance, insofar as it allows a substantial gain in the production costs of yeast [7].

The objective of the present work is to valorize the waste of dry varieties of dates *Mech-Degla* by a comparative study between these dates and the fermentation must prepared from these dry varieties.

2. Materials and Methods:

2.1. Vegetable Material:

The substrate used is formed of the waste of dates on certain varieties of common dates (*Mech-Degla*).

2.2. Methodology of Work:

2.2.1. Morphological characterization of the date:

The morphological characteristics are carried out on 10 fruits taken at random, for which are determined: Color, consistency, the dimensions of the whole fruit and its core (length and width) and the weight of the entire date, the pulp as well as the core.

2.2.2. Physicochemical characterization of the pulp:

2.2.2.1. Preparation of the date extract:

To obtain a dried variety date extract: "*Mech-Degla*", we must go through the following steps:

- Wash the fresh pulp of the date obtained with water;
- Cut the pulp into a small piece;
- In a beaker add an amount of distilled water equal to 5 times the weight of fresh prepared pulp;
- Bring the mixture to the water bath at 70 - 75 ° C for 1 hour, stirring occasionally;
- After cooling, the resulting mixture is subsequently filtered; the first filtration using a fabric (gauze), the second filtration on vacuum filter paper [8].

2.2.2.2. Determination of moisture content:

The water content of a product is directly related to the humidity of the air. It is a method of drying which consists of drying the sample at a temperature of 105 ± 2 ° C every two hours until a constant weight is obtained [9].

2.2.2.3. Determination of pH (NF V 05-108, 1970):

Based on the determination in pH unit of the potential difference between two glass electrodes immersed in an aqueous solution of the date extract.

2.2.2.4. Determination of reducing sugars:

This method based on the reduction of Fehling's liquor by the reducing sugars contained in the sample [10].

2.2.2.5. Determination of titratable acidity (NF V 05-101, 1974):

Titration of acidity with sodium hydroxide solution (NaOH) in the presence of phenolphthalein as indicator.

2-2-2-6-Determination of the ash content (NF V05-113, 1972):

The crushed date pulp is calcined at 550 ° C in a muffle furnace until a whitish ash of constant weight is obtained.

2.2.3. Preparation of must (Fermentation medium):

After manual sorting of rejects and date sorting gaps to remove particles such as stones, pebbles, plant debris,..., they are washed in tap water. After draining and coring, the pulps were cut. They are then immersed in distilled water at 75-80 ° C, at the rate of 600g pulps of waste of dates for 1.5 liters of distilled water and all carried in a water bath at 70 ° C for 45 minutes, with continuous stirring. Filtration using a gauze cloth and a pressing allows to extract the maximum of juice [11]. Nutrient needs are added to this environment such as: Magnesium sulphate 0.2 g / l, Diammonium phosphate 2.4 g / l, Ammonium sulphate 2.6 g / l, Urea 2.4 g / l and Yeast extract 7g / l. The duration of the fermentation is 14 days.

2.2.4. Analytical methods:

2-2-4- 1-Quantity of biomass:

100 ml of fermentation medium are centrifuged at 3500 rpm for 15 minutes, the base that is washed twice with sterilized distilled water.

The pellet is centrifuged with each wash and the pellet is weighed to determine the weight of biomass in fresh material then dried in an oven at 45 ° C until constant weight to determine the weight of biomass in dry matter [12].

2.2.4.2. Determination of the pH (NF V 05-108, 1970):

pH monitoring is essential for the control of microbial fermentation. Its variation provides information on the metabolic activity of the microflora.

2.2.4.3. Determination of titratable acidity (NF V 05-101, 1974):

After homogenization, the sample taken is filtered through a filter paper. 25 ml of the filtrate are taken, poured into a 250-ml volumetric flask and make up to the mark with distilled water. It is titrated with a 0.1 N sodium hydroxide solution in the presence of a colored indicator which is phenolphthalein.

2.2.4.4. Determination of reducing sugar content:

The determination of the reducing sugar content of the must by the same method mentioned in physicochemical date analyzes.

2.2.4.5. Determination of the ash content (NF V05-113, 1972):

With regard to the fermentation must, the test portion for calcination is (25 ml).

2.2.5. Microbiological analyzes:

The genera *Streptococcus*, *Lactococcus* and *Enterococcus* are highlighted on M17 medium. *Lactobacillus* and *Pediococcus* on MRS medium. Three dilution petri dishes are inoculated on M17

medium in depth on MRS medium. The enumeration of the strains is evaluated, after incubation at 30 ° C for 48 hours, in number of colonies per milliliter of sample of the must analyzed [13].

The enumeration of yeasts and molds is monitored on traditional Sabouraud medium. Incubation is for 5 days at 37 ° C. The presence of three peptones and glucose, as well as the acid pH of the medium promote the growth of yeasts and molds [14].

3. Results and Discussion:

3.1. Morphological characteristics of the date:

The physical characteristics of the date studied are given in Table N ° 1

Table N ° 1: Physical characteristics of the *Mech-Degla* date

Settings	Average value
Weight of the date (g)	6.30±0.09
Pulp weight (g)	5.2±0.2
Core weight (g)	1.12±0.14
Length of the date (g)	3.08±0.31
Width of the date (cm)	1.65±0.06
Core length (cm)	2.25±0.25
Width of the core (cm)	0.95±0.01
Weight of pulp / Weight of fresh fruit%	82.53
Pulp / Core	4.13
Length Width	1.86
Core / Datte%	17.77

The color of the *Mech-Degla* date (determined visually) varies from light brown more or less dark. The consistency of a variety is decisive for its organoleptic quality, from this point of view *Mech-Degla* is classified as dry varieties. From the results given in Table N°1, the average weight of the entire date is 6.3, while that of the pulp is 5.2; the average length and width is 3.08-1.65 respectively. The values obtained for the weight of the date and the pulp are different from those found by [15]. The latter reported for *Mech-Degla* a weight of the fruit and pulp 4.37g and 3.5g respectively.

These differences can be attributed by the instability of the water content and therefore its structure including the conditions in which the measurements are made.

The date studied *Mech-Degla* has an acceptable physical quality conferred by the criteria set by [16], [17] and [18]:

- Weight greater than or equal to 6g;
- A weight of the pulp greater than or equal to 5 g;
- A length greater than or equal to 3.5cm;
- A diameter greater than or equal to 1.5cm.

3.2. Physicochemical and biochemical characterization of the date:

The results found are given in Table N ° 2

Table N ° 2: Physicochemical characteristics of *Mech-Degla* date pulp in% of fresh weight

Settings	Average value
Water conten (%)	14.4 ±0.35
Content of reducing sugars (%)	24.23±0.06
Ash rate (%)	2±0.12
Titratable acidity (g citric acid) (%)	0.24±0.02
pH	5.62±0.06

3.2.1. Water content:

The water content of the date used in our experiment is 14.4 ± 0.35 . [19] ,[20] and [21] give contents of 13.1, 14.51 and 14.71% for the same variety. However, the majority of dates with dry consistency have water contents around 14-15%. This value favors the conservation of the dry date which can go up to a year if the conditions are favorable.

Par ailleurs, [22] ,[23] and [24] classify dates in the intermediate moisture food family, easy to store for long periods of storage at room temperature. . The date is characterized by a water content <40%

3.2.2. pH:

pH is another parameter that determines the shelf life of foods. It is one of the main obstacles that microbial flora must overcome to ensure its proliferation , [22] ,[23] and [25]. Thus, a pH of the order of 3 to 6 is very favorable to the development of yeasts and molds.

The *Mech-Degla* date has a slightly acidic pH of 5.62. This pH is detrimental to bacteria but suitable for the development of fungal flora [26] . This value is lower than that given by [20] which is 6.14 for the same variety. In the literature, [15] found that the pH of the cultivar *Mech-Degla* is 5.9, this value is comparable to our. According to [18] a date with a pH below 5.5 is of poor quality.

3.2.3. Reducing sugars:

Sugars are the most important constituents in the date. They are responsible for the sweetness of the food. Many authors, including [27], [28] and [29] agree that date sugars vary according to variety, climate, and stage of ripening. The content of the dry date: *Mech-Degla* in reducing sugars, determined by the method of Fehling is 24.23% of the fresh weight. This value is slightly higher than those found by [30, 19] with values from 16.64% to 20.92%. [31, 32] reported values of 75.68 and 52.62% for the *Alig* variety respectively.

3.2.4 .Titratable acid content:

The analyzed date has an acidity of 0.24% of fresh material. . It correlates with that found by [33] which is 0.24%.

However, our result remains much lower than that reported by [33] which found values ranging from 2.93% and 4.39% for *Degla Beida* and *Frezza* respectively. Organic acids are usually intermediates of metabolic processes. They influence the growth of microorganisms and affect the quality of preservation of products.

3.2.5. Ash content:

The value found in the date is 2% (fresh material), we can say that the latter is consistent with [8] ,[34] and [35] reported values of 1.2 and 2.69% for some Tunisian varieties. [36] give values between 1.6 and 2.0% for some Emirati varieties.

3.3. Characteristics of the medium of the fermentation (must of the fermentation):

3.3.1. Physicochemical and biochemical characterization:

After 24 hours of fermentation we observe:

- A production of gas that results in air bubbles on the surface of the must;
- The color is dark brown;
- At first the liquid is sweet.

The last days of fermentation we observe:

- Change of color; a decrease in the density due to the transformation of sugars into alcohol and formation of a deposit at the bottom of the middle.

3.3.1.1. Quantity of biomass:

Microorganisms are able to perform a wide variety of biochemical reactions that result in biomass production. That is to say, cell bodies, and by the degradation, transformation or production of organic or mineral substances , [37] .The results obtained show that biomass yields are low during the first two days of fermentation, is 0.52 g / l of dry matter. This low yield is due to low consumption of sugars during the first two days of fermentation. The amount of biomass obtained at the end of the fermentation is 3 g / l of dry matter

3.3.1.2. pH variation:

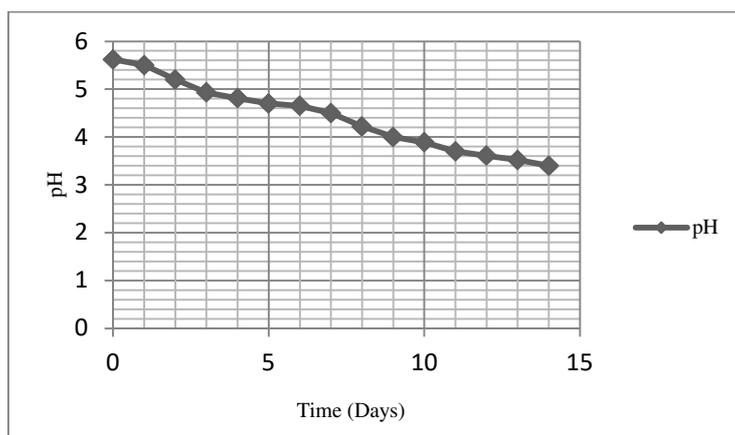


Fig.1: Evolution of the pH during fermentation of *Mech-Degla* variety dates

Note the pH of the initial reaction mixture of 5.62. As shown on the curve above, the pH of the must decreases during the fermentation to reach a value of 3.4 on the 14th day. This lowering of the pH is due initially to the diffusion of the acids contained in the date, then to the acids metabolized by the various microorganisms (mainly yeasts ...) present in the must. During fermentation, the metabolism of microorganisms induces a perpetual change of environment .Thus, the consumption of carbon and nitrogen substrates is accompanied by the production of acid or alcohol metabolites , [38].

Date must have an acidic pH. This acidic character is most often linked to the presence of organic acids. The metabolic activity of microorganisms generates the production of a multitude of products in the culture medium. This causes a variation of the pH which acts on the good growth of the microorganisms.

3.3.1.3. Evolution of titratable acidity:

The variation of the titratable acidity during the fermentation is given in the figure N °02

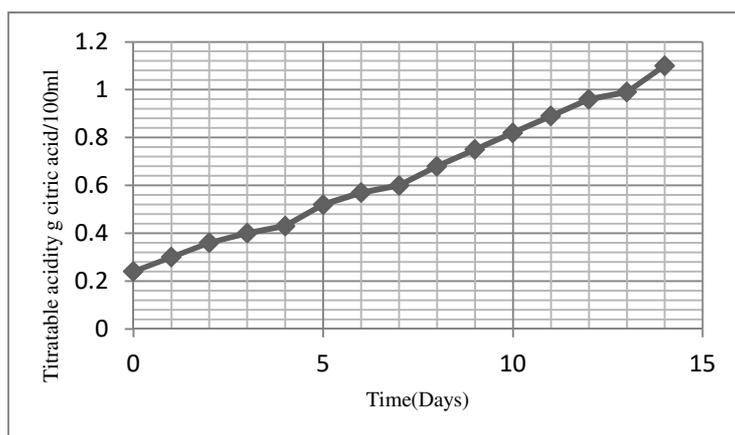


Fig. 2: Evolution of titratable acidity during fermentation of *Mech-Degla* dates.

The initial titratable acidity rate of the fermentation must be of the order of 0.24%, we notice an evolution of the acidity during the 14th days of the fermentation which reaches 1.1% (figure N ° 02). Most microorganisms are unable to grow because the acid produced lowers the pH. According to [39], the decrease in pH leads to an increase in the concentration of undissociated acids. Similarly, these authors emphasize that since the accumulation of organic acids is a function of the pH difference between the extracellular and intracellular medium, a significant inhibition would occur at a very acidic pH (pH less than 3). [38] find during fermentation, sugar consumption by yeast (glycolysis) leads to the formation of organic acids typical of fermentation.

3.3.1.4. Reducing sugar content:

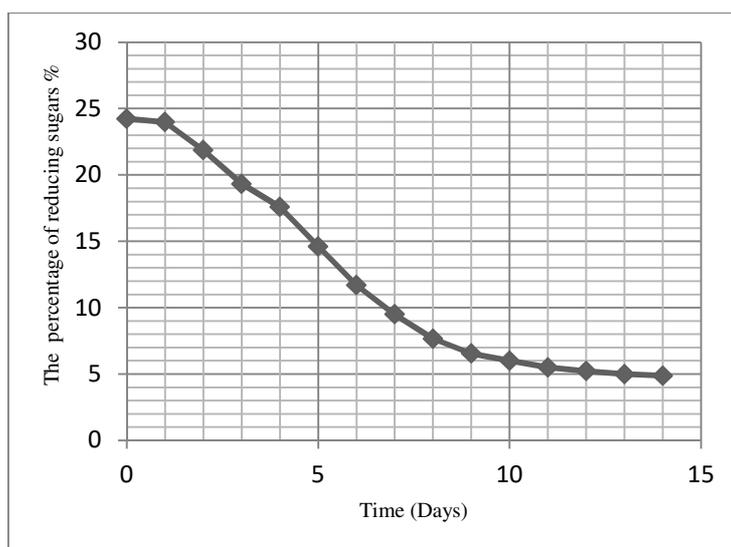


Fig. 3 : Le taux de sucres réducteur pendant la fermentation

Carbohydrate consumption by microorganisms begins as early as the first days of fermentation (Figure N°03). Their concentration varies from 23.98 to 6% after 10 days, which reflects a significant metabolic activity. After 11 days, the carbohydrate content varies little (5.5 to 4.88%) because the microorganisms do not consume them anymore. For [40] the initial pH of fermentation induces the final pH value and especially the rate of consumption of the carbon substrate: low pH slow down sugar consumption and therefore reduce productivity.

3.1.5. Ash content:

The must of the fermentation has an ash content of 0.3%, a decrease in the ash content of 2% is noted.

3.3.2. Microbiological characteristics of the fermentation must:

In the must of fermentation the bacteria are widely represented (99.53%) compared to yeasts and molds which represent respectively 0.41% and 0.04%. The selected strains are selected according to the frequency of their presence in the isolation medium. The bacteria are represented by strains S, U, X, Z, yeasts by strain Y and mold by SNC.

Results of enumeration of lactic acid bacteria:

-On the MRS medium: 6.33×10^5 CFU / ml;

-On M17 medium: 5.52×10^5 / 1 CFU / ml.

The colonies resulting from the isolations are: of variable size, of circular shape with a regular periphery; white, yellow, and cream-white:

Tableau N°03 : Identification des souches S, U, X, Z

Srains	Identified at
S	<i>Leuconostoc</i>
U	<i>Pediococcus</i>
X	<i>Leuconostoc</i>
Z	<i>Pediococcus</i>

The result of the enumeration of yeasts is 4.95×10^3 CFU / ml, a single strain Y is isolated. The colony is white, creamy, shiny, convex and smooth. The microscopic observations made on the cultures in solid medium showed the following aspects: The cells of the isolated strain are oval to round. However, some of them present on sites of their walls, invaginations, which indicates the mode of vegetative reproduction by bipolar budding and without any spore form. These results are consistent with those given by [41] for the genus *Saccharomyces* (Y).

The result of the enumeration of molds is 5.72×10^2 CFU / ml, a single SNC strain is isolated. The colony is green to brown. Microscopic observation showed septal hyphae with branched conidiophores, has a shape resembling that of a brush. Conidia are arranged in long chains, spores are round. These results are consistent with those given by [42] for the genus *Penicillium* (SNC).

The groups of microorganisms counted are lactic acid bacteria, yeasts and molds at the level of the must. These results correlate with physico-chemical analyzes, the decrease in pH during fermentation promotes the development of lactic acid bacteria, yeasts and molds that tolerate pH around 3. The increase in acidity is explained by the production of organic acids in the medium by lactic acid bacteria, yeasts and molds.

The absence of Enterobacteria in the must may be due to the relatively low pH of the must. [43] show that the production of acids during fermentation lowers the pH and thus eliminates Coliforms.

[44] showed that the use of carbohydrates by lactic acid bacteria and the production of lactic acid and acetic acid is strongly influenced by the association with yeasts and varies according to the types of sugars. The development of lactic acid bacteria is probably stimulated by the presence of yeasts that provide nitrogen compounds and factors such as vitamin B.

According to [45] it should be noted that yeasts are tolerant to lactic acid and antibiotics produced by bacteria. Organoleptically, lactic acid bacteria create an acid environment favoring the development of yeasts [44].

The big difference between the must of the fermentation and the waste of dates lies in the level of the sugar content which varies significantly during the fermentation.

Indeed, during this fermentation, yeasts use fermentable sugars that they convert into alcohol and carbon dioxide. Lactic acid bacteria which could be the same as those which ensured the lactic fermentation of the must, likely to participate in alcoholic fermentation, significant hydrolysis of non-fermentable sugars according to [46].

4. Conclusion:

The implementation of a poor quality commercial date processing industry and date waste with relatively simple biotechnological processes would perfectly meet the country's socio-economic needs. The date waste presents a very good fermentation substrate for selecting strains of industrial and medical interest. The fermentation must elaborated from *Mech-Degla* date waste is a medium rich in sugars, suitable for growing yeasts for the production of baker's yeast. According to the results obtained, date wastes can be valorized by biotechnological processes instead of their use in cattle feeding.

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