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Short Communication

Residue of dates from the food industry as a new cheap feedstock for ethanol production

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ABSTRACT

Syrup resulting from date by-products constitutes a favorable medium for yeast development, owing to its sugar composition; it was hence tested for ethanol production. Three yeasts, *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii* and *Candida pelliculosa*, were selected for ethanol production on dates syrup. In batch fermentation, the ethanol concentration depended on the initial sugar concentration and the yeast strain. For an initial sugar concentration of 174.0 ± 0.2 kg m^{-3}, maximum ethanol concentration was 63.0 ± 0.1 kg m^{-3} during *S. cerevisiae* growth, namely higher than the amounts achieved during *Z. rouxii* and *C. pelliculosa* growth, 33.0 ± 2.0 kg m^{-3} and 41.0 ± 0.3 kg m^{-3} respectively. Contrarily, only *Z. rouxii* was able to grow on 358.0 ± 1.0 kg m^{-3} initial sugar amount, resulting in 55.0 ± 1.0 kg m^{-3} ethanol produced.

1. Introduction

The date palm tree (*Phoenix dactylifera L.*) is a perennial monocotyledonous species adapted to the local conditions of arid and semi-arid areas [1]. Dates, the fruits of the date palm tree, are the major staple food in arid areas of North and Middle East Africa and the date crop plays a central role in the economy and the social life in these regions [2,3].

The date palm tree constitutes the principal source of remuneration and the basis of economy for people living in the Tunisian Sahara [2]. Today, worldwide production, utilization and industrialization of dates are continuously increasing in some countries like Egypt, Saudi Arabia, Iran and Algeria [4]. In Tunisia the number of cultivars is evaluated for

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over than 250 [5] and is currently the 10th world producer and the first exporter of dates in value. During the last five years, Tunisian production has reached an average of 120.000 tonnes per year with the dominance of the "Deglet-Nour" variety constituting about 60% of the total production [2] and 50,000 farmers are employed in this sectors. In 2011, Excess dates were 50,000 tonnes, 32% of which were from low quality dates [6].

This production progress is unfortunately accompanied by a substantial increase of loss during picking, storage, commercialization and conditioning process [7,8]. These lost dates could amount to more than 30,000 tonnes per year in Tunisia [9]. The lost date commonly named "date by-products", are not consumed by humans due to fungus and/or infestation by insects or simply due to their low quality.

Presently, by-products of dates are discarded or used in limited cases for animal feed [7,9]. Fermentation technology is one of the technologies employed for deriving value added products from by-products of dates. The various products derived from date fruit by-products are biopolymers [10,11], organic acids [12,13], amino acid [14], baker's yeast [15], probiotics [16], antibiotics [17] enzymes [18] and biofuels such as hydrogen [19] and butanol [20].

Using date by-products as a feedstock should considerably reduce the cost of production. Dates are rich in sugar ranging from 73% to 83% on dry weight basis and consisted mostly of the two inverted form, glucose and fructose [20–23]. Fresh varieties have a higher content of inverted sugars, the semi dried varieties contain equal amount of inverted sugars and sucrose, while dried varieties contain more sucrose [11].

Kasavi et al. [24] clearly established the importance of choosing the appropriate yeast strain to be used in ethanol production from biological residues; the choice will not only depend on a strain's tolerance to ethanol but also on its ability to utilize carbon sources available in agri-food residues.

The aim of this study was to evaluate the feasibility of producing bioethanol from substrate with a high level of sugars like date by-products. For this purpose, bioproduction was conducted by two osmotolerant yeasts (*Zygosaccharomyces rouxii* and *Candida pelliculosa*) and a comparative study was performed with *Saccharomyces cerevisiae*.

2. Material and methods

2.1. Microorganism

3 yeast strains were tested, the first *S. cerevisiae* well-known for its ability to produce ethanol, but this yeast is sensitive to osmotic stress; *C. Pelliculosa* has the ability to grow in media of high osmotic pressure induced by sugars or salts; and *Z. rouxii* is well-known for its capacity to grow in rich sugar environments.

The fermentative yeasts *S. cerevisiae 522D, Zygosaccharomyces rouxii* (IP 2021.92) *and Candida Pelliculosa* (IP 820.63) were obtained from the culture collection of the Pasteur Institute (Paris, France). Stock cultures were maintained on a gelified medium whose composition was (kg m^{-3}): glucose, 20; peptone, 10; yeast extract, 10; and agar, 10. In all cases, cultures were maintained at 28 °C for 24 h and then stored at 4 °C.

2.2. Inoculum preparation

A given number of drops (10) of a yeast suspension in KCl 150 mol m^{-3} was grown in 25 cm^3 of synthetic medium (kg m^{-3}): glucose, 20; peptone, 10; and yeast extract, 10; in a 250 cm^3 bottle on a rotating shaker (New brunswick, INNOVA 40, NJ, USA) at 20 rad s^{-1}, 28 °C for 18 h. After centrifugation (6000 g, 4 °C and 5 min), cells were harvested, resuspended in 25 cm^3 KCl 150 mol m^{-3} and recentrifuged in similar conditions. The suspension obtained after harvesting cells and resuspending in 10 cm^3 KCl 150 mol m^{-3} was used to inoculate culture media [25].

2.3. Raw material

By-products dates "Deglet-Nour", was obtained from a Tunisian conditioning unit of dates "ALKHALIJ". The fruits were pilled, crushed with a sharp knife and 20 g date pulp were added to 50 g of hot de-ionised water. The extraction was carried out on hot-plate at 85 °C for 45 min [26]. The juice was filtered and centrifuged at 6000 g for 30 min and then the supernatant was immediately concentrated to achieve a total sugar concentration of 720.0 ± 1.0 kg m^{-3}. The concentrated date juice was then stored at 4 °C until use.

The high sugar content allows storage without significant risk of contamination, which can be advantageous for an industrial application. However, the osmotic pressure induced by high sugar concentrations can inhibit the growth of yeasts used for ethanol production. The concentration of substrate was therefore varied from 100.0 ± 1.0 kg m^{-3} to 720.0 ± 1.0 kg m^{-3} (data not shown) and two sugar amounts were considered for this work, 17% and 36% to assess the effect of an osmotic stress.

2.4. Ethanol production medium

Dates Syrup containing 174.0 ± 0.2 kg m^{-3} and 358.0 ± 1.0 kg m^{-3} was supplemented with mineral culture medium as described previously by Djelal et al. [25]. The pH was adjusted to 6.0 using KOH 1000 mol m^{-3}. The medium was transferred into 500 cm^3 bottles with a final working volume of 300 cm^3, which were autoclaved at 120 °C for 20 min before adding the NH_4Cl sterilized by filtration on a 0.2 μm membrane (Sartorius, Goettingen, Germany).

2.5. Fermentation processes

300 cm^3 of medium containing sugar concentration of 174.0 ± 0.2 or 358.0 ± 1.0 kg m^{-3} were inoculated with 100 μL of yeast suspension. Batch fermentation was carried out in 500 cm^3 bottles on an incubator shaker (New Brunswick, INNOVA 40, NJ, USA) at 20 rad s^{-1}, 28 °C for 72 h. All experiments were performed in duplicates and samples (5 cm^3) were taken from the culture at regular time intervals.

2.6. Analytical methods

The cell density of the culture medium was measured at 600 nm (A_{600}) using a spectrophotometer (SECOMAM, Alès, France). The culture medium was then centrifuged at 6000 g,