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VALORIZATION OF BALANITES AEGYPTIACA FRUITS BY PRODUCTION OF BIOETHANOL: STUDY AND OPTIMIZATION

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Abstract
This work is part of the diversification of renewable energy sources such as biomass. The present research aims to concentrate on the valorization of desert date fruits, i.e. Balanites aegyptiaca. To the best of our knowledge, this plant has never been valorized in Algeria so far. For this, it was decided to choose one part of these fruits, namely the pulp, as a substrate for anaerobic fermentation for the production of bioethanol, which was shown to have a fairly significant added value. The results of the morphological analyses of the fruits of the desert palm indicated that the pulp represents 62.71% of the entire fruit, which is quite encouraging for the completion of the present work. It should be noted that the pulp is the most important ingredient in the fermentation process to produce alcohol. Moreover, the physicochemical characteristics of the raw material showed that the pulp of the fruits of the desert palm tree are rich in sugar, with 59.68% of total sugars and 34.18% of reducing sugars. Note also that these sugars are essential elements for alcoholic fermentation. The results obtained indicated that the optimal dilution of 1/4.4 (g / mL) gives 30% bioethanol (125 g of fruit gives 30 ml of bioethanol with 92° concentration).

Keywords: Anaerobic fermentation, Balanites aegyptiaca, Biomass, fermentable sugar, renewable energies.

1. INTRODUCTION

Over the last few years, the increase in emissions from fossil fuels (pollutants generated by combustion) and the release of greenhouse gases into the atmosphere has led to unprecedented global warming, which certainly has several negative long-term effects on the ecological systems (Jasmin, 2014; Azad et al, 2015; Dharma et al, 2016). Carbon dioxide (CO_2) is mainly generated through the combustion of fossil fuels. (Chidambaranathan et al, 2020). Consequently, it is highly urgent to think about diversifying energy sources in order to meet the increasingly growing energy needs while keeping the levels of CO_2 in the atmosphere as low as possible. It is worth indicating that the world has long faced the risk of exhaustion of fossil fuels due to their rampant consumption. (Ajiskrishnan et al, 2015). On the other hand, the possible depletion of petroleum resources in the near future and the increased socio-economic concern for the environment have led to the development of alternative sustainable fuels from cheap and environmentally friendly renewable sources. The production of biofuels from biomass could represent a reliable and efficient eco-friendly technique. It should be noted that biomass was shown to be a promising candidate for renewable energy. Biofuels, such as biogas, biodiesel, bioethanol and their derivatives, represent an attractive economic alternative that can help meet the energy needs and fulfill the ecological

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conditions. (Sindhu, Binod, and Pandey, 2016). Bioethanol can be produced from different forms of biomass, including agricultural and forestry residues, waste paper and various industrial waste streams. It can also be produced from woody and herbaceous crops, grown on underutilized land to support large-scale bioethanol production. It is worth reminding that when added to gasoline, ethanol can significantly improve fuel combustion while reducing tailpipe emissions of CO_2 and unburned hydrocarbons. (Charles and Wyman, 1996).

Balanites aegyptiaca is a forest and forage tree. It has many ecological, and mainly, medicinal features. This tree is primarily planted to fight against soil erosion. This tree species, which possesses great plasticity and considerable resistance to drought, is widespread in the Sahelian zone, as well as in Egypt, Sudan, East Africa, Saudi Arabia and India. It belongs to the monotypic Zygophyllaceae family (Fadl, 2014; Sagna et al, 2014). *Balanites aegyptiaca* is found in valleys, oases and mountainous areas, at an altitude that can reach 1000 m, and on sea coasts. This tree, which is not very demanding with regard to the nature of the soil; can grow on sandy, stony and clay soils (Elfeel, 2017; Ahmed et al, 2020). Its drupaceous fruits are rich in carbohydrates and the almond of the fruit is rich in edible oil. (Fortin, Madou, and Maynart, 1990). This forest tree is widely distributed in Algeria, particularly in the south-east, i.e. Hoggar, and in the south-west, i.e. Adrar and Bordj Badji Mokhtar. Despite its socio-economic, environmental and medicinal benefits. (Abid, Tufail and Todd, 2021), this tree has not yet attracted sufficient attention so that people would intensify its cultivation and develop its biotechnological exploitation. The main purpose of the present work consists in valorizing the fruits of the desert palm tree for the production of high-added value products, such as bioethanol, based on biotechnology processes and using the yeast Saccharomyces cerevisae as biological material. This yeast is responsible for the degradation of fermentable sugar into bioethanol under anaerobic conditions.

2. MATERIALS AND METHODS

2.1. Plant material

This This study focused on desert date fruit production sites in the region of Timiaouine in the Wilaya of Bordj Badji Mokhtar (Algeria). The dried fruits were collected from the desert date tree after full ripening (yellowish brown color) in March 2019, see figure 1.

Figure 1. Harvest of Balanites Aaegyptiaca fruits

2.2. Biological material

The biological material used in this study is the dry yeast strain Saccharomyces cerevisiae, which is employed in the production of ethanol, was stored in a cool and dry place (Boulal et al, 2016; Boulal, Kihal, and Khelifi, 2017a).

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2.3. Methodology

Preparations of dilutions

Four (04) different dilutions were prepared in g / ml, namely Dil1 (1 / 4.0), Dil2 (1 / 4.4), Dil3 (1 / 4.8), Dil4 (1 / 5.2).

2.4. Analyses performed

• Physical characteristics of desert date fruits

Parameters, such as the average weight, pulp and kernel proportions, were considered. The average weight was determined by weighing batches of 20 dried desert date fruits using a scale provided for that purpose. The masses of the fruit components were determined through weighing after pitting.

• Mineral salt rate (NF V 03.760)
• The protein content determined by the Kjeldahl method (ISO 5983-1979)
• The pH established according to standard (NF V 05-108, 1970) (AFNOR, 1982).
• Determination of the sugar content:
- Reducing sugar content. (Audigie, Figarella, and Zonszain, 1980; Boulal et al, 2013)
- Total sugar content by the Dubois method established in 1956. (Boulal et al, 2010)
- Sucrose content:

Sucrose (%) = Total sugars (%) - Reducing sugars (%)

• The number of cells was found using a Malassez Hemacytometer (Jeulin - Technical Support, Instructions - 713 441 - FR)
• Determination of the alcoholic degree of bioethanol. (Boulal et al, 2016)
• Refractive index of bioethanol (AFNOR NFISO 280: 1999)
• Determination of density of bioethanol (OIV-MA-AS2-01A: R 2012).
• IR absorption spectroscopic analysis (Analysis of constituents by infrared: ASTM D 2124)

3. RESULTS AND DISCUSSIONS

3.1. Morphological characteristics of the desert dates

The morphological characteristics of the dates under study are presented in Table 1. The values retained represent, for each characteristic, the average value of the analyses performed on 20 dates.

The results reported in the table above indicate that the desert palm fruit, ovoid in shape, has an average major axis length of about 2.98 cm and an average minor axis length of about 2.53 cm. Its color is yellowish brown; its average weight is around 7.75 grams. These results are quite different from those previously reported by Schunck (2014). These differences are due to the effects of the climatic and geographical conditions of the environment where the desert date palm is grown.

It is worth specifying that the pulp of the fruit under study is sweet and slightly sour.

Practically, during the industrial scale ethanol production, the pulp, which represents 62.71% of the entire fruit, can be removed and used in the fermentation process to produce ethanol or ethyl alcohol.

It should be noted that the results of the morphological analyses of the fruits of the desert date tree (*Balanites aegyptiaca*) showed that the pulp represents 62.71% of the total fruit. It is useful to point out that the values obtained are much larger than those found by (Elfeel and Warrag, 2006 ; Elfeel and Hindi, 2014 ; Ahmed, et al, 2020). This high percentage has attracted a large number of researchers and encouraged them to use these fruits in the fermentation process for bioethanol production. On the other hand, the physicochemical analyses indicated that desert date fruits are rich in sugars that can easily be assimilated by the human body. In addition, their pH is detrimental

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to the development of bacteria, but at the same time it does contribute to the proliferation of yeasts, which allows saying that this pH is suitable for alcoholic fermentation.

Table 1. Average values of physical characteristics of the desert dates under study

Characteristic	Average value
Fruit weight (g)	7.75
Pulp weight (g)	4.86
Seed weight (g)	3.37
Almond weight (g)	0.97
Length of minor axis of fruit (cm)	2.53
Length of the major axis of fruit (cm)	2.98
(pulp/fruit) ratio (%)	62.71
(seed/fruit) ratio (%)	43.48
(almond/fruit) ratio (%)	12.5
Color	Yellowish brown

3.2. Results of the physicochemical analysis of raw material (desert date fruits)

The results obtained are summarized in Table 2.

Table 2. Results of the physicochemical analyses of the pulp of the desert date fruit

Parameters	Results
pH	4.4 - 4.6
Humidity level (%)	41.3
Mineral salt level (%)	0.037
Protein content (%)	3,4
Total sugar content (%)	59.68
Reducing sugar content (%)	34.18
Saccharose content (%)	29.22

Table 3 clearly shows that:

- The average pH of the variety Balanites aegyptiaca varies between 4.4 and 4.6. This pH value was compared with those of some other date varieties, i.e. Deglet-Nour and Alliget Kentichi, which were determined by Elleuch et al. (2008). Their pH was found between 5.63 and 5.79.

Table 3. Results of the physicochemical analyses of the final product

Parameters	Bioethanol produced	Bioethanol 96°
pH	5.4	_
Density (20 °C)	0.823	0.789
Alcohol by volume (1st distillation)	78°	_
Alcohol by volume (after rectification)	92°	_
Yield (%)	30	_
Refraction index	1.3591	1.3594

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- The protein level is around 3,4%. These results are comparable to bibliographic data which are within the range [0.9 - 4%] relative to the fresh weight of the date. It is worth noting that (Nixon and Carpenter, 1978; Sawaya et al, 1983; Al Hooti, Sidhu, and Qabazard, 1997) reported values between 2 and 2.5%. figure 2.

Figure 2. The stages of preparation of bioethanol from fruits of Balanites aegyptiaca

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3.3. Result of the dilutions under study

This The second dilution Dil2 (1/4.4 = ratio (fruit matter-to-water) of the dilution (g / ml)) gave the highest alcohol level. For this, the present study focused mainly on the results of this second dilution.

Results of the physicochemical analysis during fermentation

The results of the physicochemical analyses of the must of desert date fruits (Balanites aegyptiaca) during fermentation are presented below.

pH

The identification of the pH is an essential step in controlling the biological activity. Figure 3 indicates that the pH decreased from 4.55 to 4.15 during fermentation. This was quite expected because the metabolic activity of yeasts caused the pH to decrease and made the medium more acidic due to the release of organic acids following the degradation of sugars. (Mehani, Boulal, and Bouchekima, 2013 ; Tadmourt et al, 2020).

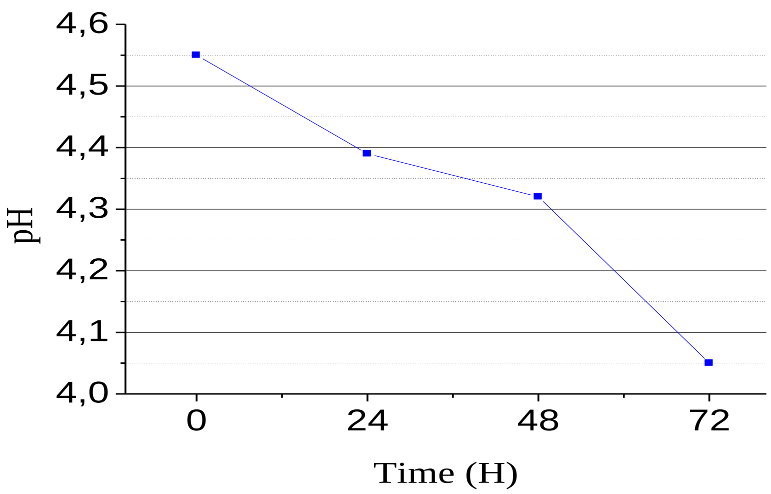

Figure 3. Evolution of pH during fermentation

It was shown that the musts of the date palm have an acidic pH. This acidic character is most often linked to the presence of organic acids whose concentration defines the degree of acidity of the must. During fermentation, some compounds undergo very little change or no change at all. Note also that other compounds including organic acids and alcohols are produced by the yeast.

Volume of CO_2 released during fermentation

Figure 4 clearly shows that during the first 24 hours of fermentation, a large amount of CO_2 is produced, which indicates that there is a good metabolic degradation during this first period. In this phase, there is transformation of sugars into bioethanol, accompanied by the release of CO_2. Then, during the last 48 hours, the volume of CO_2 released starts to decrease. (Boulal et al., 2016; Boulal, Kihal, and Khelifi, 2017a ; Ould Alhadj et al., 2018).

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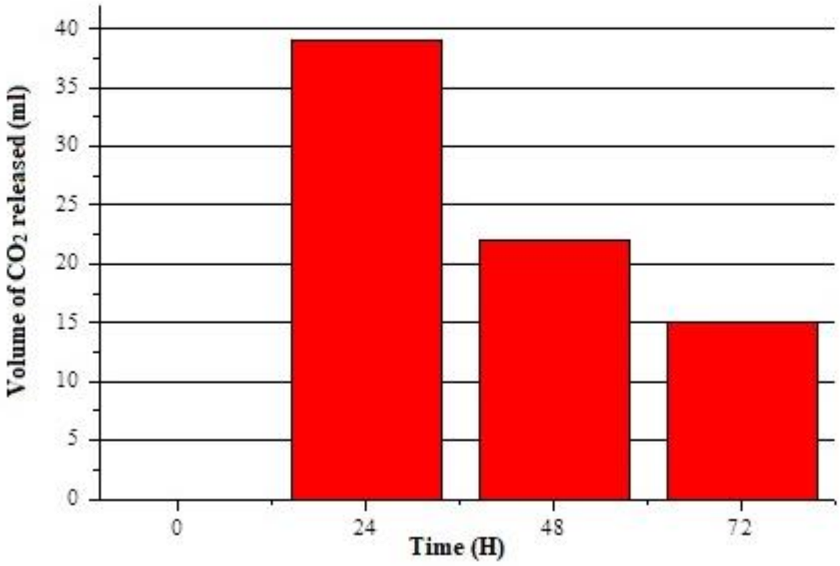

Figure 4. Evolution of CO_2 during fermentation

Mineral salt levels

Figure 5 shows a remarkable decrease in the amounts of mineral salts during the fermentation process, which means that they were consumed by the yeast during fermentation. It ought to be mentioned that mineral salts are essential elements in the fermentation process. (Tadmourt et al., 2020).

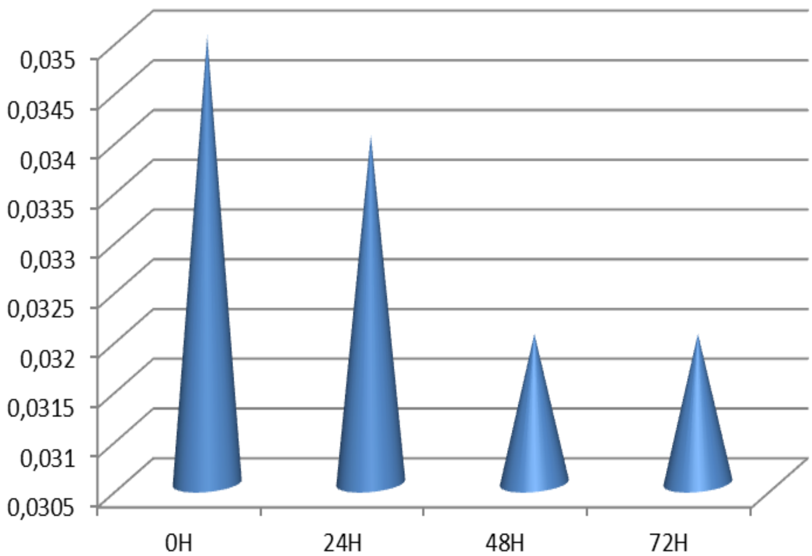

Figure 5. Evolution of the level of mineral salts during fermentation

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Density

Figure 6 shows a remarkable decrease in the must density values during fermentation, which can be explained by the transformation of glucose into alcohol and the loss of mass as a result of CO_2 emissions. (Boulal et al, 2016; Boulal, Kihal, and Khelifi, 2017a).

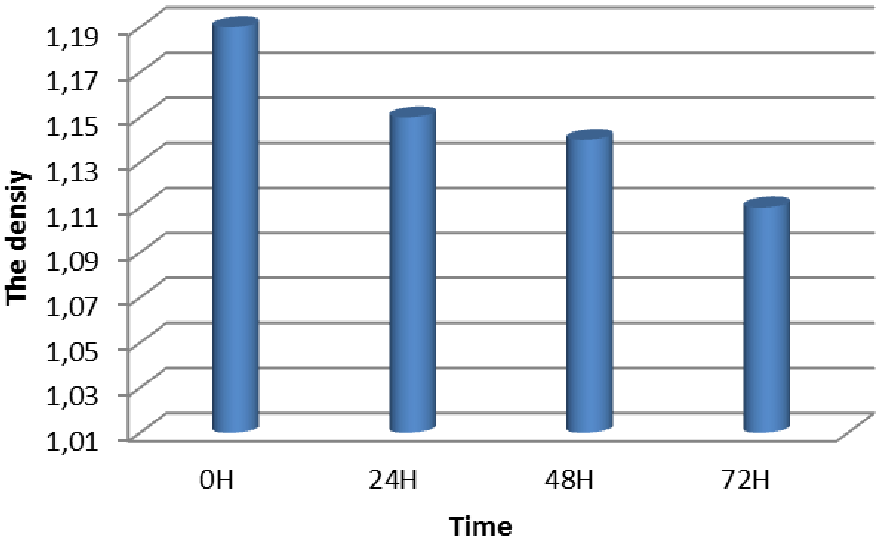

Figure 6. Evolution of density during fermentation

Refractive index

A continuous decrease in the refractive index until the fermentation stops can be clearly seen in Figure 7, which means that there is a direct link between the refractive index and density decrease.

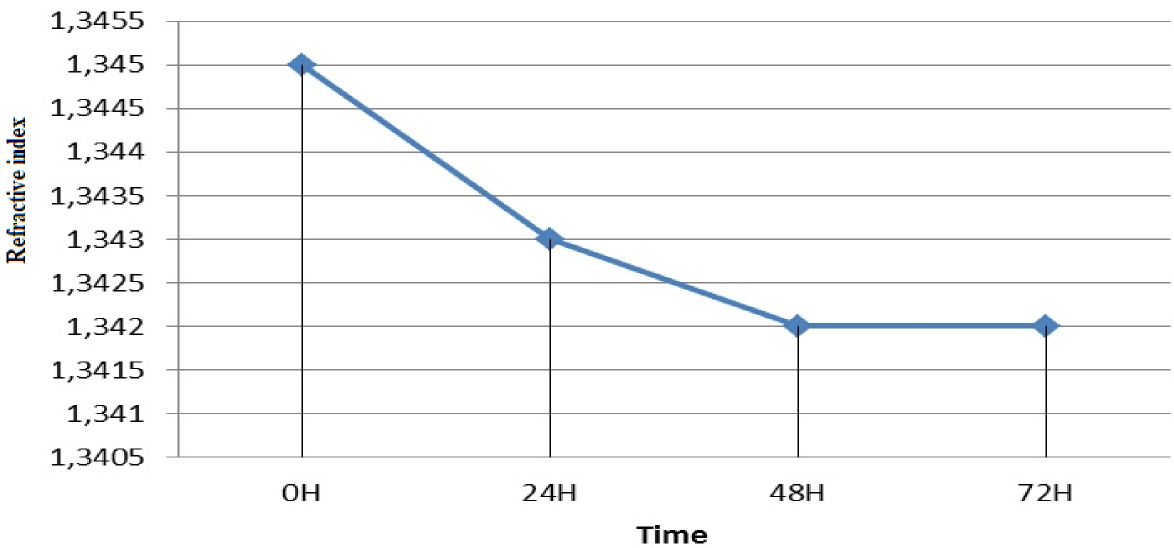

Figure 7. Evolution of the refractive index during fermentation

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Growth of yeast cells

The change in the number of yeast cells during fermentation can clearly be observed in Figure 8. In fact, it is easy to see that the yeast Saccharomyces cerevisea follows a microbial growth kinetics that is described by the following three phases:

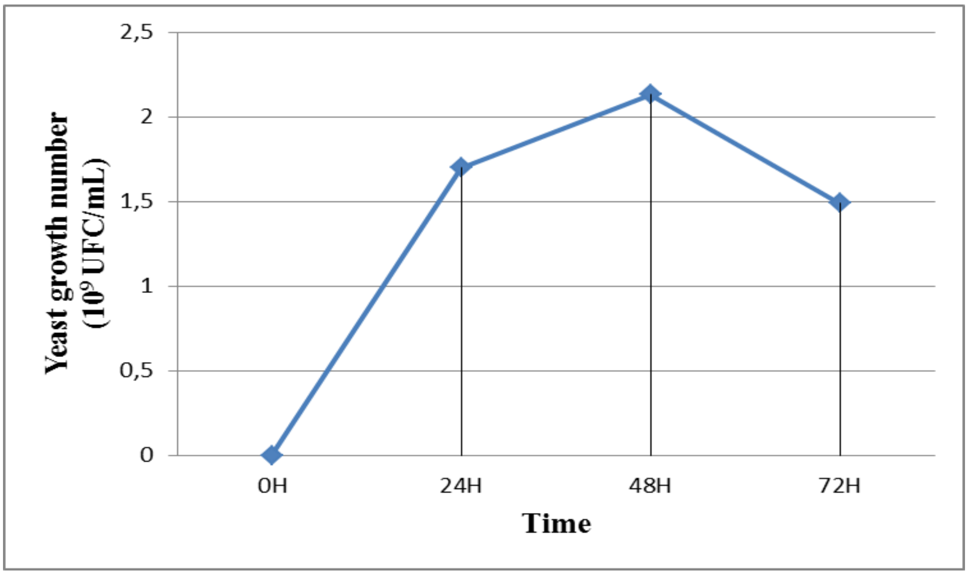

Figure 8. Evolution of yeast growth during fermentation

a. Growth phase

This phase takes place during the first 24 hours. It is characterized by a rapid and progressive growth rate, where the yeast population reaches a maximum value of 2.12 × 109 cells / µL for *Balanites aeygptiaca*. This strong growth rate is due to cell multiplication. The development of new cells depends on the consumption of sugars, nitrogen and minerals,

b. Stationary phase

The nitrogen, which is required for the construction of new cells, reaches its minimum amount due to the stopping of yeast growth,

c. Decay phase

On the third day (after 72 h), the concentration of viable cells decreases due to cell lysis that is caused by the unfavorable conditions in the medium.

Reducing sugars

Figure 9 shows a very significant decrease in reducing sugars content during the first 48 hours of fermentation. This decline in reducing sugars is probably due to the conversion of fermented sugar into ethanol. It should be noted that the production of ethanol gradually decreased due to the decrease in sugar levels and to the accumulation of toxic compounds as well. After 72 hours, it was observed that the sugars were not completely consumed by the yeast, which was probably due to the fact that the yeast Saccharomyces cerevisiae stopped growing as a result of the accumulation of toxic substances. (Boulal et al, 2010). This indicates that, first, the fatty acids formed turned the yeast toxic and, second, the alcohol in the medium became inhibitory.

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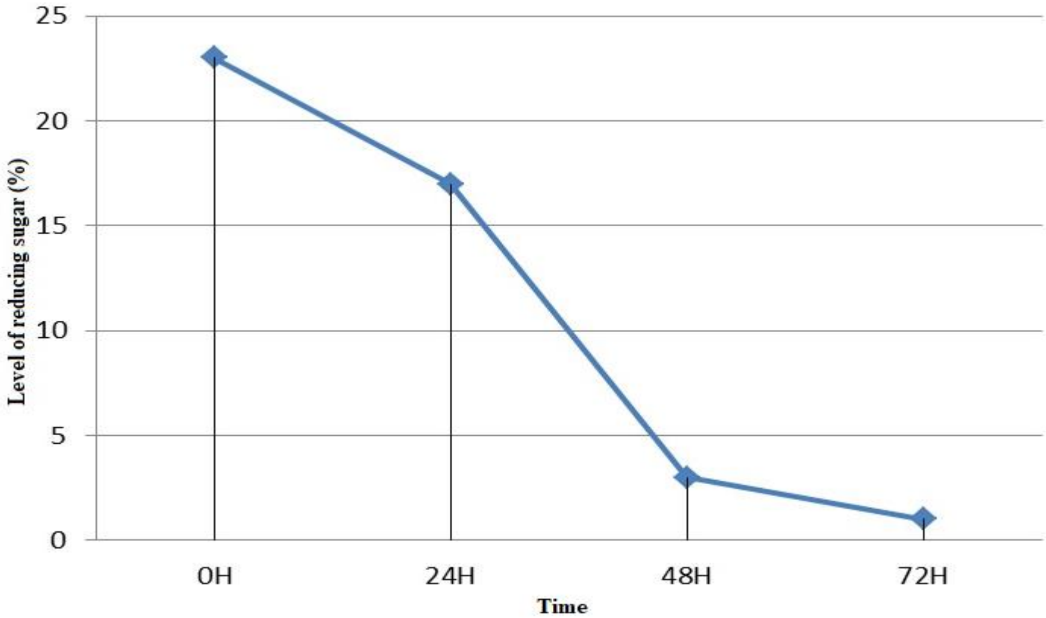

Figure 9. Evolution of the content of reducing sugars during fermentation

Alcohol content

Figure 10 clearly shows that the alcohol content increased during fermentation and the fermentation kinetics of alcohol production are related to the sugar content in the fruit must.

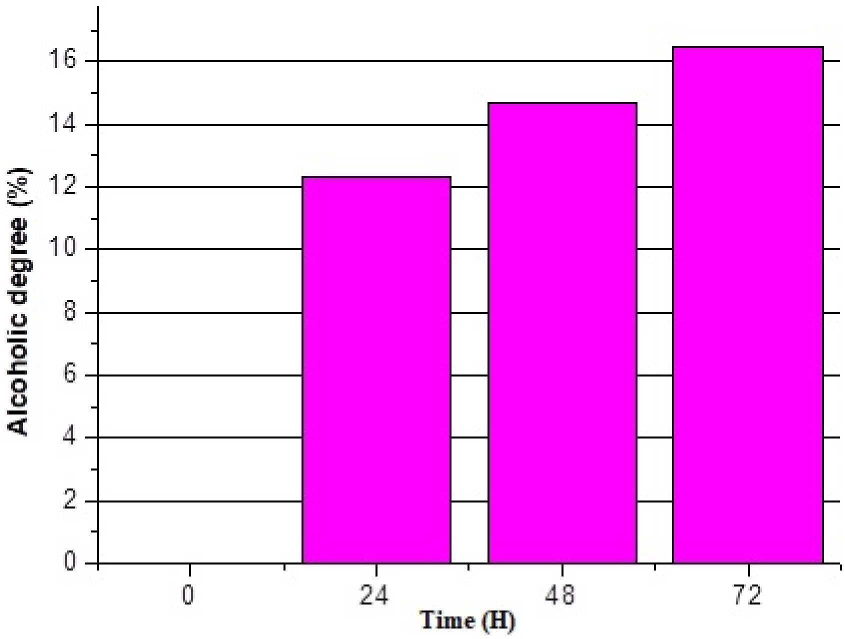

Figure 10. Evolution of alcoholic degree during fermentation

3.4. Results of the physicochemical analysis of the final product

The alcohol produced at the laboratory level turned out to be quite volatile, flammable, and clear; it has a pungent odor.

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The results of the physicochemical analyses of the bioethanol produced from the desert date tree are summarized in Table 3.
Infrared spectrophotometric analyses of the ethanol obtained through the distillation of the most of the desert date tree fruit

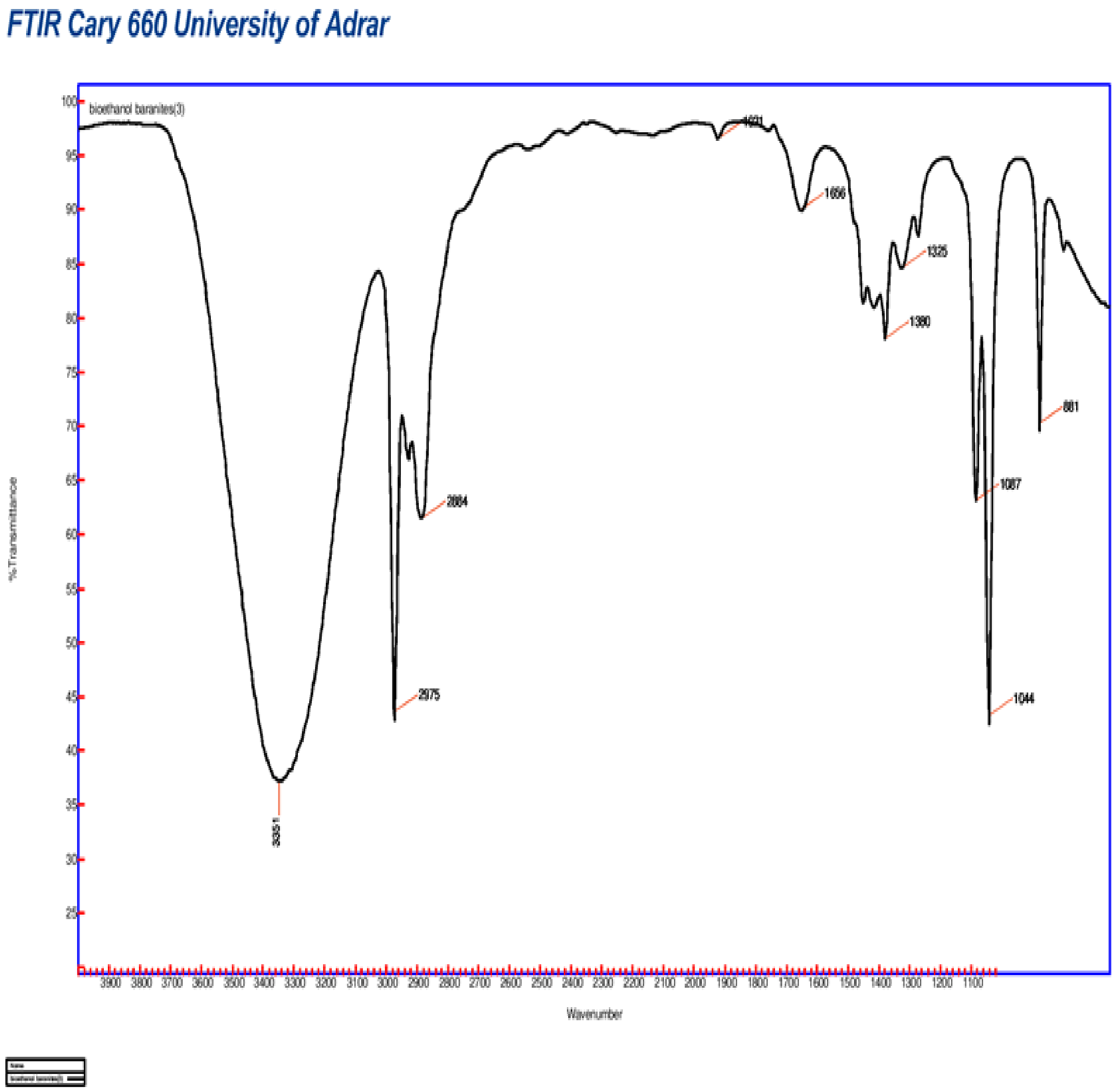

Figure 11. Infrared spectrum of bioethanol obtained from Balanites aegyptica

Figure 11 shows the vibrational signatures of the bands obtained by infrared spectrophotometry of ethanol products. The vibrations of the different bands that appeared within the spectrum are illustrated in Table 4. The groupings correspond to the band vibrations.

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Table 4. Groupings corresponding to the valence vibration

Band vibrations	Group	Compound
3339.08	C-OH	Alcohol
2974.29	C-H	Alcane
2888.12	C-H	Alcane
1380.91	C-OH	Alcohol
1087.06	C-OH	Alcohol
1044.53	C-OH	Alcohol

3.5. Weight yield

The weight yield is defined as the ratio between the quantity of alcohol produced and the quantity of fruit (with stones) used. (Boulal et al, 2010).
The calculated yield corresponding to Balanites aeygeptiaca is such that:

55g → 100%
16.47 mL → x

This means that each 125g of fruit produces 30 mL of alcohol with a 92° concentration.
The study of the fermentation of fruit must allowed noting that:
- The alcoholic level (alcohol by volume) evolves progressively during the fermentation process in parallel with the degradation of sugars. It could reach 79° at the end of fermentation,
- After rectification, the alcohol by volume obtained is 92°.
- The characteristics, such as density, pH and refractive index, of the resulting bio-alcohol are close to those of pure ethanol,
- The resulting yield is around 23.35%.

The present work allowed showing that the sweet and dry fruits of desert date tree (Balanites aegyptiaca) can be valorized. Indeed, instead of losing them in nature or feeding them to animals, they can be recycled to produce ethanol, after fermentation and alcoholic distillation.

4. CONCLUSIONS

The findings of the present study allowed concluding that it is possible to produce bioethanol at the local level from the fruits of desert date tree (*Balanites aegyptiaca*) which are widely available in the region. In addition, it would be desirable to extend the present work with the view to further investigate the valorization of the desert dates for the production of ethyl alcohol on the industrial scale in order to meet local and national needs.

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