Production of Bioethanol from the Waste of three Varieties of Vitis Vinifera Grapes (green, red and black)

Ahmed MEDIANI[1*], Amel MEHREZ[2], Oum Elkheir BENDAHOU[2], Akil LOUMANI [1], Cherif TIGANI[1] and Abdelkrim DJABER[1]

[1]Unité de Recherche en Energies Renouvelables en Milieu Saharien, URERMS, Centre de Développement des Energies Renouvelables, CDER, Adrar 01000, Alegria

[2]Department des Hydraucarbures et Energies Renouvelables, Faculté des Sciences et de la Technlgie, Université d'Adrar, Adrar, Algeria

[*]Corresponding author; Email: medianiusto@yahoo.fr

Article Info

Article history:

Received May 09,2022
Revised, August 15, 2022
Accepted, November 20, 2022

Keywords:

Bioethanol
Grapewaste
Fermentation
Saccharomyces cerevisiae
renewable energies
micro-organism.

ABSTRACT

Algerian market waste from grapes represents an interesting source for the recovery of market waste into renewable energy, while eliminating this waste to contribute to the protection of the environment within the framework of sustainable development. This work studies the production of bioethanol from three varieties of grapes existing in the Algerian market. This waste has undergone physico-chemical and microbiological analyzes before being fermented by the yeast *Saccharomyces cerevisiae* at the laboratory level. The results obtained by these analyzes confirmed to us that the grapes contain a significant quantity of sugar and also confirmed the presence of some microorganisms such as mephilic total germs.
Finally, the ethanol was recovered by distillation, making it possible to reach 91% alcohol. The results show that alcohol from grape waste has great potential for the production of ethanol by referring to that from waste from the potato processing industry.

I. Introduction

Algeria is one of the most prominent grape-producing countries at the Arab level, occupying the second place after Egypt with around or more than 502978 tons per year, which represents a huge production of this vital wealth with an agricultural area of more than 68 thousand hectares for grapes, what has distinguished it with this characteristic is the suitable and ideal climate for the adaptation of the growth and fruiting of grape-producing vines (FAO, 2019) [1].
The favorable climatic and pedological factors explain the richness of the Algerian viticulture grape varieties. Viticulture is everywhere throughout the Algerian country in the West: Tlemcen, Sidi Bel Abbés and Ain Témouchent are the main vine-producing towns in the East Skikda and Bejaia, and in the center are the hills of Sahel, Blida, Médéa, Mitidja and Kabylia [2]. Covering an area of 56,000 ha in 1998, the Algerian vineyard rose to 75,000 ha in 2017. But it only ranks 22nd worldwide [3]. Grape production has also improved considerably, with a 75% increase between 2010-2017 and 2000-2009 [4].

The grape harvest is exposed to rapid damage due to factors of transport, packaging, storage, even climatic conditions and the nature of this fragile and easily damaged fruit, especially in the states of the Great South in general and l he state of Adrar in particular because it is characterized by intense heat during the period of harvest and flight of this harvest (from the end of spring to the beginning of autumn).

The objective of this study is the use of waste from three varieties of *vitis vinifera* grapes (green, red and black) as a fermentation substrate for the production of bioethanol by anaerobic bioconversion of date waste in the presence of dry yeast *Saccharomyces cerevisiae*. In addition, a comparison will be made as to the

performance of the three varieties. It is worth noting that our country imports between 30,000 and 50,000 hectoliters of ethyl alcohol per year to cover its various needs [5].

II.Material

II.1. Plant material

In this work, three types of spoiled grapes available on the market were used with less value (waste of three varieties of *vitis vinifera* grapes (green, red and black)

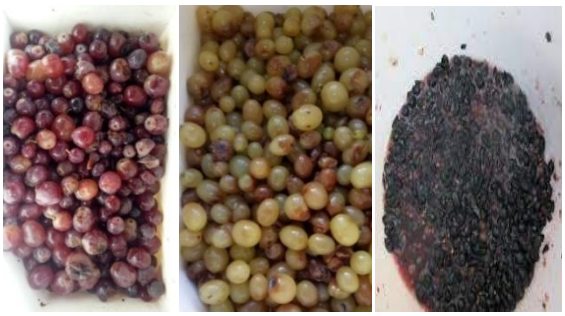


Figure 01: The biomass used *Vitis vinifera* (red, green, black)

II.2. Biological material

The yeast species *Saccharomyces cerevisiae* selected in this work is the one marketed in Algeria (dry baker's yeast) [6].

II.2. Physico-chemical analysis methodologies

II.2.1. Determination of moisture content

The dry matter content is determined by the A.O.A.C method (1980)

Dry empty crucibles in an oven for 15 minutes at 105°C; Tare the crucibles after cooling in a desiccator; Weigh 5g of sample in each crucible to an accuracy of 0.001g and place them in the spray oven at 105°C for 24 hours; Remove the crucibles from the oven, place them in the desiccator and after cooling, the operation is repeated until a constant weight is obtained (using the drying time of 30 minutes).

Expression of results: $$H\% = (M_1 - M_2)/ M_2 * 100$$

H%: Humidity **M_1:** Mass of the crucibles + material before stoving **M_2:** Mass of the assembly after curing

The dry matter content is calculated as follows: **Matière sèche %= 100 − H%**

II.2.2. Determination of ash content and organic matter (AFNOR, 1972)

The quantity of sample (05g) is introduced into an oven for 24 hours at 105°C then into a muffle furnace (Nobertherm) for 4 hours at 550°C.

$$MO\% = (M_1 - M_2/M_1) * 100$$

MO%: Organic matter **M_1:** Mass of the crucible + test portion **M_2:** Mass of the crucible + ashes

The ash content **(Cd)** is calculated as follows: **Cd = 100 − MO (%)**

II.2.3. Determination of water activity

Water activity is measured by Aw meter by enclosing approximately 2 to 5g of sample in a component cell of an AWX 3001EBRO screen electronic device. After a minimum of 1h30, the reading is made on the display screen.

II.2.4. pH determination (AFNOR, 1970)

Place a quantity of fruit in a beaker and crush it manually; Determination of the pH value by the pH meter.

II.2.5. Determination of sugar content (Dubois 1956 method) [6]

a) Preparation of the sample: 1g of product (grape: red; green and black) was mixed with 300 ml of distilled water and 3g of $CaCO_3$, then the mixture was heated for 30 min until boiling with continued stirring afterwards filtered. After cooling the mixture, complete with distilled water up to 1 liter (1000 ml) of the solutions, after adding a small amount of lead acetate afterwards filtered.

b) Dosage: After the 2nd filtration, we had a filtered extract from which we took 01ml which we mixed with 01ml of phenol (5%) and 05ml of concentrated H_2SO_4 with continued stirring. The tubes are kept for 5 min at 100°C after staying in the dark for 30 min. Then we read the O.D related to the wavelength 490 nm by UV-VIS spectrophotometer .

We took 5 ml of our product, to which we added 5 ml of HCl (2N); We heat to 100°C in a water bath for 30 min and after cooling we take 1 ml of this extract and proceed to the dosage of the sugars contained, as for the reducing sugars already presented as well as the reducing sugars obtained by hydrolysis of sucrose, therefore these are the total sugars.

Sucrose = (Total sugars – Reducing sugars) *0.95

Of which: 0.95 is a correction factor (the correlation between the experimental and the calculation model)

II.2.6. Determination of Brix rate and refractive index

Clean the blade of the device using drops of distilled water and Joseph paper; Calibrate the device with distilled water whose refractive index is equal to + 1.33; Clean the refractometer blade using Joseph paper; Place a few drops of syrup on the refractometer blade and set the darkened chamber circle clear in half; Read the results on the eyepiece scale, taking into account the ambient temperature, so the value displayed must be corrected according to the temperature; Clean the refractometer blade, always using Joseph paper; An analysis by a refractometer was carried out to know the sugar content dissolved in this solution.

II.3. Microbiological charatersitics

II.3.1. Preparation of culture medium

The culture media used for the count of total germs, Coliforms, Yeasts and molds are respectively the following:

- Standard Plate Count Agar (PCA). [7]
- Bile Crystal Violet Lactose Agar (VRBL).
- Sabouraud agar (OGA).

II.3.2. Numbering technique (UFC) [7]

The technique consists of four consecutive steps: Serial preparation of tenth dilutions; Seeding.; Incubation; Enumeration of colonies and interpretation of results [8].

a) Preparation of the dilutions

- Thinners:

- Buffered peptone water: preparation of the stock suspension. - Tryptone-salt: Preparation of decimal dilutions.

- Procedure:

Preparation of the mother suspension: Weigh in a stomacher bag a mass m (25) representative of the juice of two (02) varieties of grapes. Add an amount of thinner equal to 9* m. This quantity is measured in mass with an uncertainty of ± 2% [9].

Preparation of decimal dilutions: Dilutions are always carried out under aseptic conditions [10].

b) Inoculation and incubation

In the case of counting yeasts, molds, the inoculation was done on the surface. Gold for the count of total germs and total Coliforms was made in depth.

1. Surface inoculation **2.** Deep inoculation

Finally leave to cool until complete gelation of the medium.

The incubation of the inverted boxes was done in an oven thermostated at a temperature and a well-determined time.

c) Reading and interpretation

Reading is done by visual counting. In all cases, only dishes containing 20 to 300 colonies are used. The number of colonies obtained per box makes it possible to go back to the starting microbial concentration, and this according to:

• When a dish has no colonies, we conclude: "less than 1 germ per ml"

• When the number of colonies is for all the dilutions lower than 20, we conclude: "less than 20 germs per grams or per ml"

• When the number of colonies is between 20 and 300, we calculate: the average number of colonies represented as a weighted average from two successive dilutions, as follows:

$$N = \Sigma c / 1.1\ d$$ **(NF V 08 – 102, 1998) [10]**

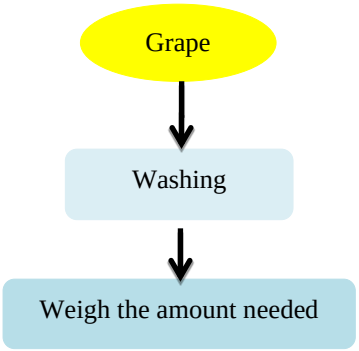
Where:

c: number of colonies in the dishes of two successive dilutions

d: dilution rate corresponds to the first dilution retained

III. Steps of bioethanol production

III.1. Biomass pretreatment protocol for the extraction of grape pomace (substrate)



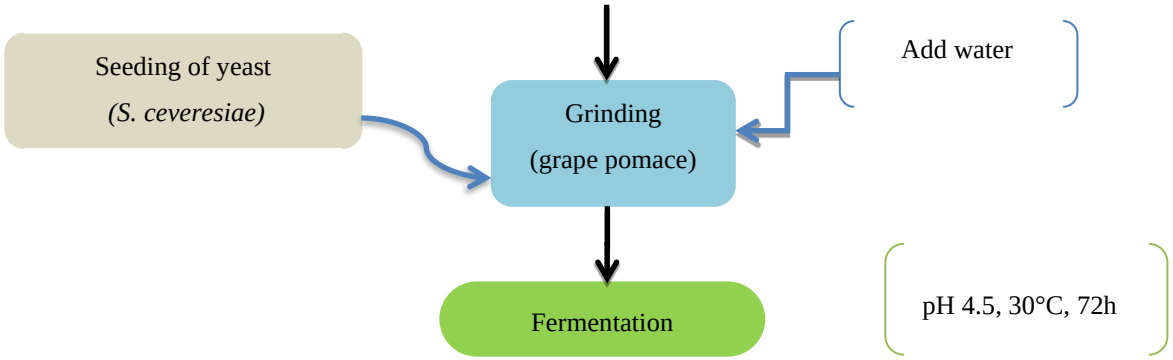


Figure 02: Stages of Grape Fermentation

III.2. Description of the bioethanol manufacturing process

Has gone through three (03) stages:

1) Distillation of wine; 2) Purification of bioethanol; 3) Dosage of alcohol

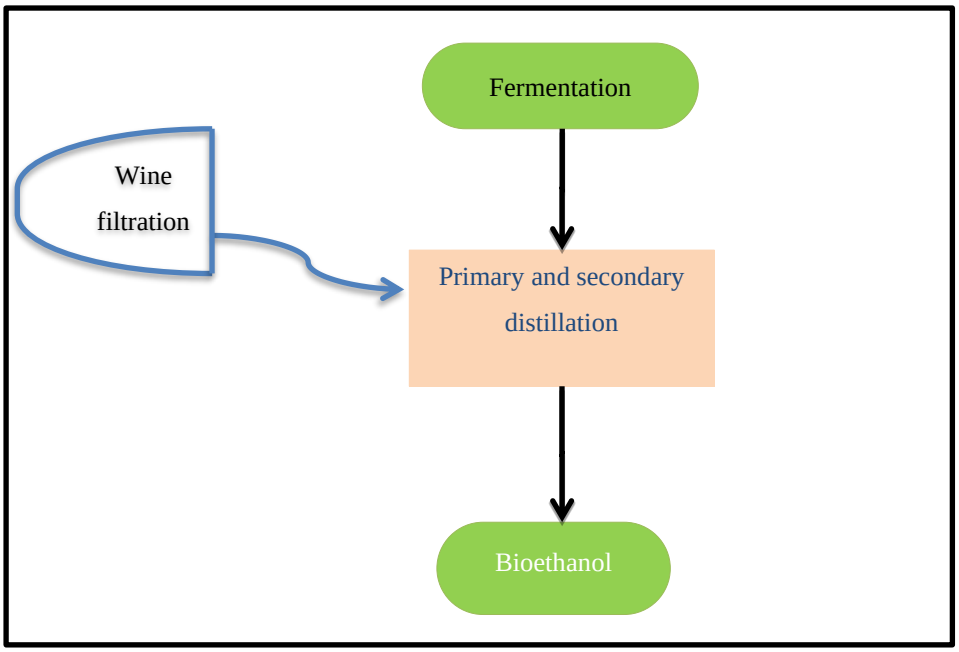


Figure 03: The different stages of the bioethanol production process

In order to monitor the progress of the fermentation, we proceed every 24 hours. Samples to carry out physicochemical analyses. At the end of each time, the grape pomace is filtered and then distilled to extract the ethanol. The distillation temperature is around 78°C.

- **Determination of pH:** The pH is measured using a pH meter with stirring.
- **Determination of density:** Density is the ratio of the weight of a certain volume of a determined body to that of the same volume of pure water under the same temperature conditions [11] [12].; we determined the density of our catch test using a pycnometer (10 ml).

$$D = m/v$$

D: the density in g/ml; **m:** the sample mass in g; **v:** the volume of the pycnometer (10mL).

- **Dosage of the alcoholic degree:** The dosage of the alcoholic degree during the fermentation is carried out by aerometry. The method consists of distilling the alcoholic juice, then measuring the alcohol content of the distillate at room temperature using an alcoholometer (graduated from 0 to 100°) [12] [14].
- **Assay of total sugars (Dubois method, 1956):** This assay method makes it possible to determine the concentration of sugars (total sugars) in the product.

Monosaccharides → acid medium furfuralic derivatives → phenol colored compounds. [15].

IV. Results and Discussions

IV.1. Physicochemical analyzes before fermentation

In this part, we interpreted the results for the three (03) varieties of grape waste in the following tables:

Table 01: Physicochemical properties of waste from three Vitis vinifera grape varieties (red, green and black)

Type of analysis	Red grape	green grape	Black Grappe
Humidity rate (%)	27.65	29.34	16.681
Dry matter content (%)	72.35	70.66	83.318
Ash content (%) (From 72%)	26.837	26.301	-
Organic matter (%) (From 72%)	72.163	73.699	-
pH	5.04	4.92	4.90
Titratable acidity (%)	1.6	1.8	-
Water activity (AW)	0.999 à22.5°c	0.903 à 20.1°c	0.885 à 20.3°c
Brix (%)	15.7	13.5	18.2
Refractive index	1.3654	1.3621	1.3694
Total sugars (g/l)	141.6	133.9	229.7
Sugar percentage (%)	1.9	1.8	-

According to Table 01, waste from conventional grapes generally has water contents varying between 16% and 29% depending on the species and the variety. The water content of black grapes takes a low value 16.681%, but the red grape is 27.65%. This value is relatively average compared to the water content of green grapes which brings the value of 29.34%, this value makes it possible to increase the water activity to 0.903 (at a temperature of about 20.1°C) which is responsible for weathering reactions and ensuring proper storage of waste. Grape sugars vary according to the variety considered, the climate and the stage of ripening. The results reported by different authors depend in part on the method used. Nevertheless, all agree that the reducing sugar content of green grape waste is 0.80 nanometers with a titratable acidity of 1.8% before alcoholic fermentation. This value is very important for the production of bioethanol. The average pH varies between 4.92 for green grapes, 5.04 for red grapes and 4.90 for black grapes.

We note that the sugar concentration varies between 133.9 g/l and 229.7 g/l, knowing that the black grape variety contains the highest concentration (229.9 g/l).

IV.2. Microbiological analyzes before fermentation for the two varieties of grapes

Table 02: Results of microbiological analyzes of green grapes

	Culture medium		
Variety 01 (green)	**PCA**	**OGA**	**VRBL**
10^{-1}	++++++	–	–
10^{-2}	–	–	–
10^{-3}	–	–	–

Tableau 03: Results of microbiological analyzes of red grapes

	Culture medium		
Variety 02 (red)	**PCA**	**OGA**	**VRBL**
10^{-1}	–	–	–
10^{-2}	+++	–	–
10^{-3}	–	–	–

The two tables 02 and 03 represent the explanatory results of the microbiological analyses. On the one hand, we note a very high quantitative presence (+++) of total germs in a dose of 10^{-1}ufc/g of grape juice in PCA medium for the green grape variety, on the other hand there is a total absence (-) of these micro-organisms in this dose for the other grape variety. On the other hand, the total germs are present in limited quantity (+++) in a dose of grape juice of 10^{-2} ufc/g for the red variety, but they are absent (-) in this dose for the green variety.

In a dose of 10^{-3} ufc/g, total germs do not exist (-) for both varieties of grapes. We also note that yeasts and molds, coliforms are completely absent in all the different doses (for the two varieties of red and green grapes).

IV.3. the alcoholic degree after the first distillation of the wine

After fermentation of the grape waste, we calculated the alcoholic degree for each variety knowing that 1 represents red grapes, 2 represents black grapes and 3 represents green grapes.

Table 04: The alcoholic degree after the first distillation of the wine

Sample	Total quantity	Amount of distillate	Alcoholic degree
1	2750 ml	900 ml	20%
2	2650 ml	325 ml	45%
3	2500 ml	950 ml	20%

From this table, we notice that the type of sample and the total quantity of wine used influence the D.A (alcoholic degree). The latter takes the best value of 45% in 325 ml of distillate for black grapes, the two other red and green varieties represent only 20% of alcoholic degree in quantities of distillate vary between 900 ml and 950 ml successively.

So, we see that the total quantity of wine and the type of sample are very important parameters to obtain an excellent D.A (alcoholic degree).

IV.4. Comparative study between the bioethanol produced and commercial alcohol

We compared between bioethanol produced to commercial ethanol by several parameters in the following table:

Table 05: Characteristics of bioethanol

	bioethanol produced				
	red	**Black**	**green**	**characteristics of bioethanol**	**Commercial bioethanol**
Density	0.964 at 09°	0.844 at 09°	0.881 at 09°	0.81 at 0.9 °	0.82
T°=boiling	76	74	79	**73**	**73**
pH	7.36	7.36	7.42	**7.35**	**7.34**
Refractive index (°C)	1.37185	1.3721	1.3718		1.3616 at 25.2°C 1.36394 at 20°C
Alcoholic degree (%)	82	88	75	**91**	**96**
Color	Transparent	Transparent	Transparent	Transparent	Transparent
Odour	Spicy	Spicy	Spicy	**Very spicy**	**Very spicy**

At the end of the production of bioethanol, we made a comparison between commercial alcohol and bioethanol produced from plant biomass; it is the waste of three varieties of grapes.

The first remark to make is that the bioethanol obtained in the three cases has a density of 0.84-0.96 with a pH varying between 7.36 and 7.43; these values are close to that of commercial ethanol. In addition, our product actually has a pungent alcohol smell and is highly flammable (Figure 04).

For the three alcohol samples, the flame is very intense, long-lasting and reminiscent of that obtained with gasoline.

Figure 04: Flame obtained after the combustion of bioethanol

On the other hand, we note that the alcoholic degree of the bioethanol produced is 88% for the black grape variety, on the other hand the D.A of 75% and 82% for the other successive green and red varieties. But after the recovery of the whole produced bioethanol will be increased up to 91%.

These results are expected since it was previously demonstrated that diluted grape juice was well suited to the development of *S. cerevisiae*. It can therefore be concluded that grape waste represents a good raw material for extracting a juice used as a fermentation substrate.

It can be said that the bioethanol produced at the laboratory level had the following characteristics: volatile, flammable, limpid, and pungent and has a very high alcohol content, therefore it complies with international standards (INRS, 1997).

V. Conclusion

In this present work, the grape from the waste of the Algerian market was used as raw material for the production of bioethanol. The choice of three varieties of grape is justified by its abundance in Algeria and also to know and compare the alcoholic degree of each variety with the alcoholic degree of commercial ethanol. It was then thought that it would be wiser to produce ethanol from grape waste.

The results of the present experimental work on the fermentation of grape juice show that a highly flammable alcohol is obtained which can be used as fuel. The alcoholic degrees of bioethanol obtained varied between 82% and 91%. The highest value corresponds to the black grape juice which seems to be very rich in compounds necessary for the development of yeasts.

The *S. cerevisiae* type yeasts used in this work were chosen for several reasons: availability, rapid growth, resistance to certain contaminants, fermentative power and above all the ability to consume most sugars.

Finally, common grape residues are considered a good raw material from which a very good substrate for alcoholic fermentation can be extracted. A fermentation optimization study could lead to an optimal Brix value which would give very good quality bioethanol.

Acknowledgements

We would like to express our sincere thanks to the research unit in renewable energies in the Saharan environment, especially the solar drying team for the reception in the laboratory, the provision of the equipment and product necessary for the realization of this study.

References

[1] http://www.aoad.org/GrapsGuide2021.pdf

[2] LERY, 1982, L'agriculture au Maghreb G.P. Ed. Maisonneuve et Larose. pp284-286.

[3] OVI, 2018 - *World vitiviniculture situation. OVNI statistical report on world vitiviniculture*. Édit. International Organisation of Vine and Wine, Paris, 25 p.

[4] MADR, 2019 - *Le renouveau agricole et rural en marche : revue et perspectives*. Édit. Ministère de l'Agriculture et du Développement Rural, Alger (Algérie), 58 p.

[5] Touzi. A, 1997, Valorisation des Produits et Sous-Produits de la Datte par les Procédés Biotechnologiques. Rapport de Synthèse de l'Atelier. Technologie et Qualité de la Datte, CIHEAM. Options Méditerranéennes. Pp. 214.

[6] LOUMANI, A. (2011). Etude microbiologique et hygiénique du yaourt fabriqué et commercialisé dans l'Ouest Algérien (Doctoral dissertation, Université d'Oran1-Ahmed Ben Bella).

[7] Loumani, A., Larbi, A. A., Mediani, A., Chaouch, W. B., Moungar, H., Tigani, C., ... & Bekada, A. M. A. (2020). Experimental Measurement of Isothermal Sorption, Microbiological and Physicochemical Analysis of Dried Tomatoes Cultivated in Adrar, Algeria. Journal homepage: http://iieta. org/journals/ijdne, 15(5), 721-728

[8] Menad, N., Bensoltane, A., Loumani, A., Maghnia, D., Slimane, N., Chougrani, F., & Cheriguene, A. (2012). Antimicrobial Activity of Probiotics on Swiss Mice. Journal of Food Science and Engineering, 2(7), 382.

[9] AKIL, L., FATIMA, B., & AHMED, B. ENUMERATION, ISOLATION AND IDENTIFICATION PHENOTYPIC THERMOPHILE LACTIC ACID BACTERIA ISOLATED FROM DIFFERENT FERMENTED MILK COLLECTED IN ALGERIA

[10] A. B. DuBois, S. Y. Botelho, J. H. Comroe, and others, "A new method for measuring airway resistance in man using a body plethysmograph: values in normal subjects and in patients with respiratory disease," J. Clin. Invest. vol. 35, no. 3, pp. 327–335, 1956.

[11] GUIRAUD J.P. (1998). Microbiologie alimentaire. Ed. Dunod : 576p.

[12] Néji, O. (1983). Techniques de dénombrement des bactéries. Manuel pratique de microbiologie générale, Tunisie, 205-215.

[13] Association Francaise de Normalisation Indice de classement: V08-102XP. XP NFV 08 102 December 1998. (06/2003)

[14] GUIRAUD J.P. (1998). Microbiologie alimentaire. Ed. Dunod : 576p.

[15] Boulal A., Kihal M. et Khelifi CH. (2017). Bioethanol and yeast strain production from common dates. LAMBERT Academic Publishing (LAP). Juin 2017. ISBN 978-3-330-32696-5. www.get-morebooks.com.