Intensive Bioethanol Production Using Date Stems by-Products and Natural Sugars

Samira, Elbey; Fatma Zohra, Zoubiri; Selsabila, Zara; Rachida, Rihani
Laboratoire Phénomènes de transfert (LPDT),Université des Sciences et de la Technologie Houari Boumediene (USTHB),Bab-Ezzouar, Algiers, ALGERIA.

Souhaib, Zerrouki[*+]
Laboratoire Biotechnologies, Ecole Nationale Supérieure de Biotechnologie Taoufik Khaznadar. Nouveau pôle universitaire Ali Mendjli BP E66, Constantine, ALGERIA.

ABSTRACT: *Date Stem By-products (DSB) have been used as agricultural feedstock to perform bioethanol production in a batch bioreactor. To study the effect of an optimal mixture of fruit hydrolysates (20%dates, 20% Figs, and 20% sugar beet) on ethanol yield, different parameters have been followed up, including pH, total sugars, and ethanol yield. The optimal ethanol concentration was found for date stems by-products +20% dates, it was about 19.38 g/L after 72h fermentation process, in such case, the higher initial sugar concentration is close to 204.5 g/L, and the pH dropped from 4.5 to 4.21, while it was only about 18.48, 13.08 and 4.72 g/L for DSB+20% sugar beet, DSB+20% Figs, and DSB, respectively. The maximum bioethanol production rate (Rm) was about 2.633 (g/L/h) in the case of DSB+20% dates, which is higher compared to the other selected mixtures. The effect of some physical properties on the addition of ethanol/ETBE (Ethyl tertiary-butyl ether) to super-premium gasoline has been studied. It was found that the addition of 20% ethanol or ETBE to super-premium gasoline increased the Octane Number from 96 up to 99.3 and from 96 up to 99.8 in the case of DSB+20% dates and ETBE, respectively.*

KEYWORDS: *Date-stems by-products (DSB); Fermentation; Natural sugars; Bioethanol; Octane number; ASTM distillation; Sulphur content.*

INTRODUCTION

In recent years, research has increasingly focused on the development of alternative transportation biofuels. The use of byproducts-derived biomass as renewable energy sources remains a very promising option compared to fossil fuels [1].

Faced with the inevitable disappearance of petroleum reserves as well as the adverse effect on the ecology related to the increase of a fossil fuel supply, there is a growing interest in advanced renewable energies. Biofuels are a reliable option for the future and can be considered as the base of the sustainable supply of clean and reliable energy and environment-friendly. Biofuels can be produced from several types of feedstocks, including agricultural residues, processing industrial residues, forestry industry, recycled by-products, energy crops, and municipal solid

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* *To whom correspondence should be addressed.*
\+ *E-mail: s.zerrouki@ensbiotech.edu.dz*
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wastes [1]. These raw materials must be available in bulk quantities and their use does not compete with food crops.

Thus, bioethanol and biodiesel are increasingly recognized as the main biofuel sources in the transportation sector. These fuels can be used in different percentage blends with conventional liquid fuels to form a mixture of biofuel. They can be injected into internal combustion engines without significant modifications [2].

Fuel bioethanol is one of these promising biofuels due to its benefits such as smoke-free burning and low flash point [3], it has similar properties to petrol. The exhaust gases generated by the combustion process are less reactive with sunlight than those produced from fossil fuels, leading to further strengthening of the protection of the ozone layer [4]. Besides, this product is hygroscopic, which cannot be transported by pipeline because the moisture contained in bioethanol can result in the corrosion of equipment.

Fuel bioethanol is usually sold in Brazil as a 18-27.5% blend with gasoline [5]. It can be mixed with diesel and biodiesel in diesel engines to reduce particulate matter emissions [6].

Bioethanol can be obtained through the fermentation of sugarcane and corn as the main raw materials used worldwide [7,8]. Besides, other renewable raw materials are also being increasingly tested such as coconut mesocarp, oil palm empty fruit bunches, hardwoods, apple wood, and many more [9-12].

A significant limiting factor in transforming many raw materials into bioethanol concerns the costs of these biomasses and this is why the research was focused on using an abundant and low-cost feedstock. Ethanol can also be extracted from seaweed [13].

Dates are considered edible sweet fruit mainly cropped in arid areas. Consumers are looking to dates for their health benefits as they are high in natural carbohydrates, fat, and dietary fiber. The use of date fruits ensures the long-term existence of these valued cultural arid landscapes. The main producers' countries are Egypt, the Kingdom of Saudi Arabia, Iran, Algeria, Iraq, Pakistan, Sudan, and Oman with an annual yield of 1690959; 1541769; 1283499; 1 151 909; 735353; 543269 and 465323 tones, respectively [14]. Date processing processes generate significant amounts of by-products including recyclable materials.

Lignocellulosic biomass is recognized as a renewable, available, and cheaper resource that can be processed for energy generation, which constitutes a direct substitute for conventional fossil fuels in the transportation sector.

The choice of raw material depends on its high availability, its chemical and physical properties, and the processes that lead to a high percentage of bioethanol in the long term. Most importantly, this waste will not compete with human food products. They are referred to as second-generation renewable fuels.

To improve the yield of bioethanol production several methods are often researched to pretreat the raw materials such as chemical, physical, biological, and combined pretreatments [15]. The main purpose of the pretreatment step is to make the complex carbohydrate polymers more accessible to hydrolysis by breaking up complex materials into shorter chains.

The aim of this study consists of using date stems as a potential feedstock for the ethanol production process. This residue is considered an interesting energy material due to its availability and abundance in many arid regions which could reveal better financial management. The effect of natural sugars from some fruits (Figs, sugar beets, dates) has been assessed through the conversion of sugars from date stems into bioethanol by using the Strain of VdH2 of *S. cerevisiae*. A Gompertz model was used to predict bioethanol production. Furthermore, characterization of bioethanol-gasoline blends was performed in terms of distillation curve, sulfur content, and octane number.

EXPERIMENTAL SECTION

Experimental set-up

Ethanol Fermentations have been performed in the stainless-steel bioreactor (Fig.1) at cylindrical-shaped and conical-bottomed. This fermenter has 15 cm in diameter and 35.5 cm in total height. The entire fermenter volume is 5 liters with a substrate volume of 3 L. The mixing was induced using a Rushton-type turbine of 10.4 cm diameter, which was located at a distance about 1/3 above the bottom. This impeller is regarded as a good bubble breakup and gas dispersion ensuring a good mass and heat transfer [16, 17].

The impeller speed was about 150 rpm, inducing a radial flow inside the fermenter. Impeller-to-cylindrical bioreactor diameter ratio was about 1.43. The fermenter was supplied with an integrated heating element and a thermostat to maintain consistent the temperature of

Table 1: Date stems by-products (DSB) charactristics

characteristics	value %(w/w)
Ash	4.73
Dry matter	23.75
Cellulose	40.53
Hemicelulsoe	16.54
Lignin	14.54
Proteins (g/g)	2.64
Total sugars (g/g)	13

Table 2: Elemental composition of DSB

Element	Value %(w/w)
C	45.60
O	47.62
Cl	1.60
K	5.18

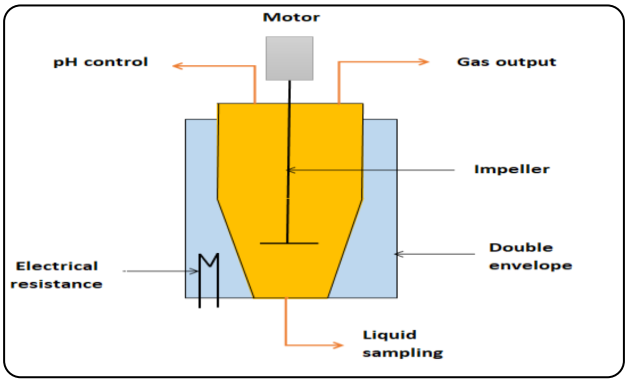

Fig. 1: Experimental setup

Fig. 2: Date stems

fermentation. On the top head of the reactor, several nozzles have been inserted in the tight-fitting lid and serve to measure several parameters via sensors such as pH, temperature, and withdrawn liquid samples for analysis. The bioreactor was fitted with a purge valve in the lower zone of the conical arrangement.

The fermentations have been conducted at an initial pH of 4.5. The initial pH adjustment has been carried out by adding NaOH or HCl (respectively at 0.2 N and 0.1 N).

Raw material

The date stem by-products (DSB) were collected fresh from the various markets located in the Biskra region, south of Algeria (Fig.1). DBS biomass was first washed and then dried at about 45±2°C °C for 24h by using a laboratory oven (MEMMERT model UM 100) to remove the amount of moisture from a wet DSB. The DSB was milled, homogenized, and sieved using a screening machine (CISA model BA-200N) to achieve an average particle size upper than 63 µm. The DSB composition was carried out according to the National Renewable Energy Laboratory's (NREL's) Laboratory Analytical methods [18]**.** The characteristics of DSB are given in Table 1. It can be seen that DSB contains a significant amount of cellulose (40.53 % of dry matter), a high level of lignin (14.54% of dry matter), and a low ash content (4,73% of dry matter).

The elementary analysis provided by DSB elementary composition is given in Table 2. In addition to the high percentage of carbon and oxygen, DSB shows the presence of macroelements such as chloride and potassium. This biomass can be regarded as a required nutritional medium for *S. cerevisiae* (Strain of VdH2) growth and appears as a promising feedstock for bioethanol production. The biomass particles have been placed and stored in sealed plastic boxes at 4°C until subsequent use. 20% of natural sugars contained in some fruits have been also added to date stems, such as Figs, sugar beets, and dates.

To enhance the D-xylose extraction from hemicellulose and D-glucose from cellulose, the oxidative hydrolysis of DSB was performed using a 5% (v/v) hydrogen peroxide solution at a temperature of 75°C for 4 hours of reaction.

Analytical methods

The characterization and the chemical composition of date stem byproducts with respect to the proportion of cellulose, hemicellulose, and lignin has been carried out under NREL method [19]**.** For carbohydrate characterization, the phenol-sulfuric acid method has been used (*Dubois et al.* 1956) [20]**.** Proteins and lipids concentrations

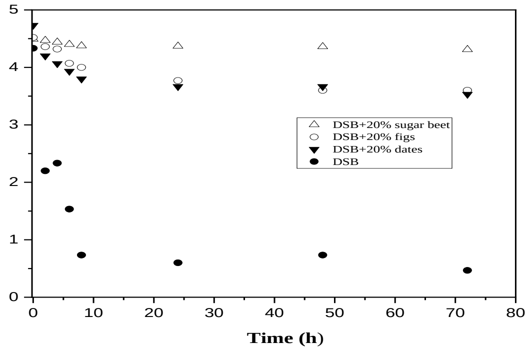

Fig. 3: pH evolution during ethanolic fermentation

have been determined respectively using the (*Lowry et al*) [21] and (*Bligh* and *Dyer*) [22] methods.

The bioethanol composition was measured using gas chromatography (GC) (Agilent 7890A). The GC was fitted with an Agilent HP Column (30 m, 320 µm i.d., 0.25 m). Nitrogen has been used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was set to 62 °C with a rate increase of 25 °C/min until it reached the temperature of 120 °C. The injector and detector temperatures were set to 220°C and 300 °C, respectively.

Characterization of bioethanol and its blends were carried out following the American Society for Testing Materials (ASTM) procedures, in the Sidi Rezine refinery and Naftal of Dar El Beida, laboratories. A distillation temperature of samples was performed according to ASTMD86. The octane number was measured by the Research Octane Number (RON) (ASTM D2699).

To withdraw cell mass, the samples taken from the fermenter has been centrifuged. The supernatant contains sugars as well as ethanol identified and quantified using high-performance liquid chromatography. All analytical values were calculated from triplicates and average results are provided.

The modified Gompertz model

The experimental data of bioethanol production over time have been modeled using the modified Gompertz model [23], according to the following equation:

$$B(t) = P \cdot exp\left\{-exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \qquad (1)$$

Where B *(t)* is the bioethanol concentration production during the fermentation time t, (in g/L), *P* is the bioethanol concentration potential (in g/L), R_m is the maximum bioethanol production rate (in g/L/h), and λ the lag phase duration (in hours).

The model performance has been evaluated using statistical parameters. The correlation coefficient (R^2) and Root Mean Square Error (RMSE) have been calculated using equation (2) [24].

$$\text{RMSE}=\left(\frac{1}{\text{m}}\sum_{\text{j}=1}^{\text{m}}\left(\frac{\text{d}_\text{j}}{\text{y}_\text{j}}\right)^2\right)^{\frac{1}{2}} \qquad (2)$$

where m is the data pair number, *j* is the *j*th values, *y* is the measured values and *d* is the deviations between measured and predicted values. Kinetic constants of P, λ and R_m were estimated using non-linear regression with the help of MATLAB R2014b software.

RESULTS AND DISCUSSION

pH evolution

pH is an important parameter in yeast activity, it indicates the activity of hydrogen ions in the medium. Fig 3 shows the temporal evolution of the pH of different mixtures based on a date stems (DSB) hydrolysate to which 20% of natural sugars (sugar beet, Figs, and dates) have been added. It can be seen that the pH level decreases gradually for all cases during the first 10 hours. Then, it tends to fluctuate between 4.5 and 4.21 for DSB mixtures with natural sugars. While, it is more pronounced for DSB hydrolysate, the pH value dropped from 4.5 to 3.6. In such a case, the reactor medium was acidified due to the carbon dioxide formation and the accumulation of volatile fatty acids. These observations are in agreement with those of previous studies [4, 25]. Most *Saccharomyces cerevisiae* strains can grow at a range of pH tolerance between 2.50 and 8.50, but they are acidophilic organisms that develop even in an acidic environment [26]. The yeast exhibits a pH value at which growth is optimal, it varies between 4.00 and 6.00, depending upon such factors as the temperature, and the strain of yeast [27]. Therefore, to ensure high bioethanol production, it is necessary to keep the stability of the ethanolic fermentation process, i.e., the pH value within 4.5- 6.5 [28].

Total sugars uptake

The effect of natural sugars on total sugars evolution during DSB ethanolic fermentation process is given in Fig. 4. For different natural sugars selected, it can be seen that the uptake of the carbohydrates decreased exponentially

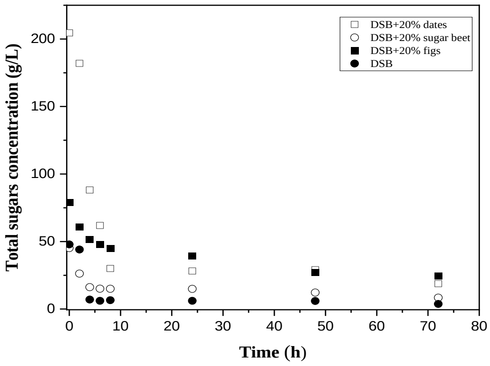

Fig. 4: Effect of natural sugars on total sugar consumption during DSB ethanolic fermentation process

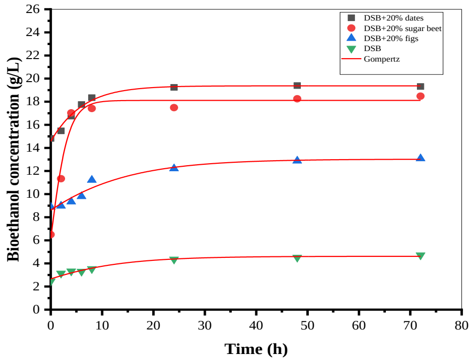

Fig. 5: Effect of natural sugars on bioethanol production from DSB

at the beginning of the fermentation, i.e. during the first 10 hours and it seems more pronounced and fast in the case of DSB+20% dates. The analysis of the reaction rate constant (k) confirms this finding, it has been found the values of 1.34 10^{-1} h^{-1}, 2.7 10^{-1} h^{-1}, and 1.7910^{-1} h^{-1}, 2.3410^{-1} h^{-1} for DSB+20% Figs, DSB+20% dates, DSB+20% sugar beet, and DSB, respectively. The total sugar values reached are 24.39, 18.76, 8.44, and 4.75 g/L after 72h of fermentation based on the yield efficiency corresponding to the values of 69, 90.83, 81.31, and 90.07%, respectively, in the case of DSB+20% Figs, DSB+20% dates, DSB+20% sugar beet and DSB, respectively. The use of natural sugars from dates remains a promising nutrient medium depending upon the level of glucose content in the dates which provides good conditions for yeast adaptation and growth [29], thus improving the efficiency of sugar conversion into bioethanol. The results indicated that the addition of natural sugars from different fruits improves bioethanol production concerning carbohydrate assimilation.

Bioethanol production modeling

The effect of natural sugars on bioethanol production from DSB is depicted in Fig. 5. The experimental and modeled profiles are given in the same Fig. It can be seen that the bioethanol profiles increase over time for date stems (DSB) hydrolysates containing different levels of natural sugars. It can be seen that the bioethanol synthesis is triggered during the first hours of the fermentation process for all selected systems. This is due to the fast consumption of total sugars by *S. cerevisiae* cells during 10h that ferment sugars to ethanol [30].

The results from this study showed that the ethanol concentration increases over time till it reaches a steady state after 24h of the ethanolic fermentation process. This is due to the slow up of sugar consumption and the medium is more acidic after inducing ethanol and carbon dioxide [4]. The highest ethanol concentration was reached for DSB+20% dates, it was about 19.38 g/L after 72h fermentation process, while it was only about 18.48, 13.08, and 4,72 g/L for DSB+20% sugar beet, DSB+20% Figs, and DSB, respectively. These results are higher than those found by (Karagöz) [30], who found that the alkaline peroxide pretreatment of rapessed straw produced 5.73 g of ethanol.

The use of the DSB mixture with fruits results in three and four times higher ethanol concentrations compared to the amount produced from only DSB raw material. This bioethanol production can be ascribed to the initial amount and uptake of sugars by yeast growth [31]. Due to its high sugar content, DSB+20% date constitutes an attractive raw material for bioethanol production.

Table 3 summarizes the kinetic parameters of the modified Gompertz model. The maximum bioethanol production rate (Rm) was more important in the case of DSB+20% dates compared to the other selected mixtures, it was about 2.633 (g/L/h), while it was only about 0.503, 0.155, and 0.132 (g/L/h) for DSB+20% sugar beet, DSB+20% Figs, and DSB, respectively. Modeled lag phase duration, was significantly reduced in all selected mixtures, the values found ranged from 0.1 to 0.2 h, which indicates that the bioethanol production was beginning immediately on the first hour of the ethanolic fermentation process. This suggested that 0.2 h was required for the *S. cerevisiae* cells adapt to the medium and ferment sugars to ethanol.

A good agreement was observed between the modified Gompertz model and experimental data, giving a good fit with $R^2 > 0.94$, and the RMSE value fell within the range of 0.081–0.097 in all cases.

Table 3: Kinetic constants parameters of Modified Gompertz model

	B (t)(g/L)	P(g/L)	R_m (g/L/h)	λ (h)	R^2	RMSE
DSB+20% sugar beet	19.31	19.312	0.503	0.20	0.985	0.081
DSB+20% dates	19.38	19.438	2.633	0.10	0.965	0.084
DSB+20% Figs	13.08	12.722	0.155	0.20	0.946	0.097
DSB	4.72	4.526	0.132	0.12	0.956	0.099

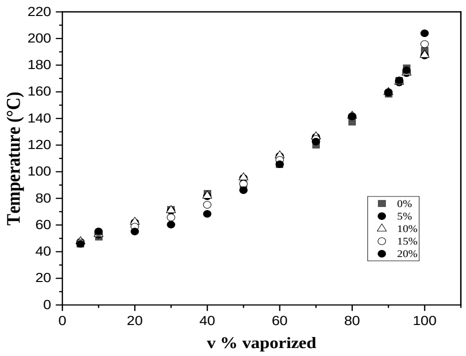

Fig. 6: ASTM Distillation curves of super premium gasoline and its blend with bioethanol

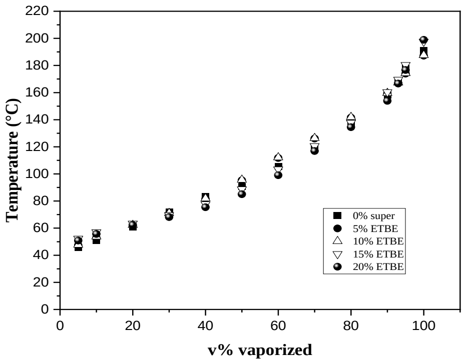

Fig. 7: ASTM Distillation curves of super premium gasoline and its blend with ETBE

Physical properties of ethanol and its blend with super-premium gasoline

Distillation curves

The fuel distillation range can significantly affect the engine performance. It can be separated into different fractions in various steps: heating, vaporizing, collection, and condensation of fuel. The separation can be conducted for different petroleum products such as gasoline, kerosenes, gas oils, etc. The test was performed under atmospheric pressure according to the standard ASTM D86 with 100mL of ethanol-super premium gasoline (SPG) and ETBE- super-premium gasoline. The rising fuel vapors were collected into a measuring cylinder after their condensation through a coolant. A comparison between experimental data of vapor temperature distribution versus the cumulative volume of liquid fractions is depicted in Figs. 6 and 7. For all ethanol/ETBE-gasoline blends tested, it can be seen that the temperatures increased with the increase of the mixture vaporized fraction with respect to their composition and their boiling points. It can also be seen that the addition of ethanol or ETBE to premium gasoline did not affect the vapor temperature of the first drop of condensate which corresponded to the initial boiling point (IBP). As the heating of fuels proceeded, more mixtures of hydrocarbon vapors with increasing boiling points were collected whatever the super-premium gasoline blends. For light fractions, the addition of a small amount of ETBE or ethanol has a slight effect on the gasoline fuel distillation when compared to the neat gasoline distillation. At 10% boiling temperature, the distillation temperatures reached were 50.99°C (super premium gasoline); 52.78°C (super-premium gasoline + E5); 53.15°C (super-premium gasoline +E10); 54.42°C (super-premium gasoline +E15); 55.19°C (super-premium gasoline +E20). While the temperatures are slightly higher when adding ETBE up to 15%, it was about 53.75°C (super-premium gasoline +10%ETBE), 57.07°C (super-premium gasoline +15% ETBE) and 55.69°C (super-premium gasoline +20% ETBE). The light fractions are easy to spray, which is an important need for combustion in internal combustion engines. Between 40 and 60% vaporized volume, T50 was affected by the addition of oxygenated additives. In the case of super premium gasoline, ethanol from DSB+20% dates and ETBE increased the boiling point from 95.59 to 95.63°C for a volume fraction of 15% up to this value the temperature decreased to the values of 86.11 and 85°C for 20% in the case of super-premium gasoline+E20 and super-premium gasoline+ 20% ETBE.

For all blends, the FBP will not exceed the temperature of 203.93°C, which did not surpass the limit (205°C) fixed in the ASTM D86 standard. The addition of ETBE/bioethanol to gasoline involved lower volatility for

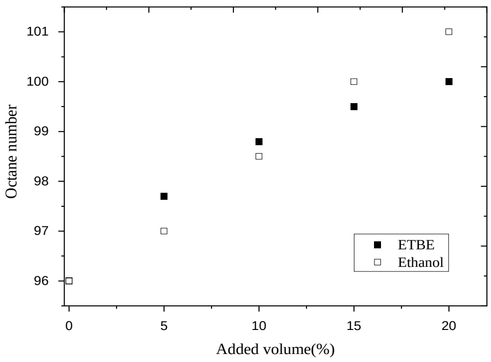

Fig. 8: Octane number curves of super premium gasoline and its blend with bioethanol or ETBE

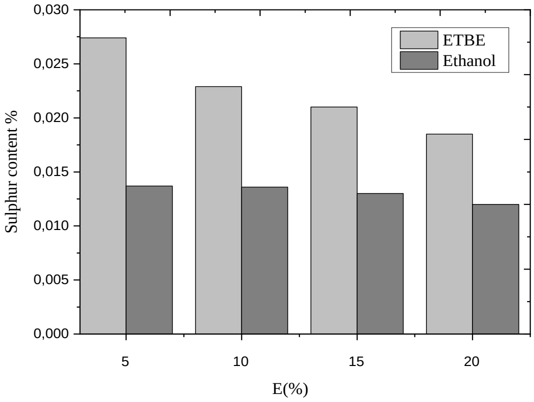

Fig. 9: Sulphur content of super premium gasoline and its blend with bioethanol or ETBE

lighter fractions and higher volatility for medium and heavy fractions [32].

Fuel volatility and vaporization are important for reducing any vaporization of the fuel and thus keeping the minimal fuel consumption. If the fuel was volatile leading to a high risk of a loss of both power and efficiency due to the formation of a vapor lock. It was important to identify the fuel temperatures at the start and the end of distillation due to their significance in relation to the burning characteristics.

Octane number

A fuel mixture needs to meet the specification for volatility and octane. Both proprieties are most important for gasoline. Octane rating is a unit of measurement established by the automotive industry to determine the antiknock quality of fuels. It was considered the performance indicator of a gasoline's resistance to auto-ignition and knock during compression in the combustion chamber of the spark-ignition gasoline engine. In fact, the thermodynamic efficiency of the engine cycle increased with the increase in compression ratio. But there was an upper limit for compression ratio, the limit beyond which the efficiency decreased by generating a metallic knocking or pinging sound. Research octane numbers for gasoline and ethanol blend are depicted in Fig. 8. It can be seen that the super-premium gasoline may appear to have different octane numbers when blended with DSB+20% ethanol or ETBE at different proportions (E5, E10, E15, and E20). When 20% of the ethanol produced from DSB+20% dates or ETBE was added to super-premium gasoline, the RON increased from 96 up to 101 and from 96 up to 100, respectively. The ethanol from DSB+20% dates with gasoline blends provided a relatively slightly higher-octane rating compared to ETBE. The presence of isoparaffins and alcohol in gasoline fuels enhanced gasoline octane levels (anti-knock). The effect of adding ethanol at low ratios to gasoline has been studied by some researchers, who found that the increase of ethanol content in the gasoline led to the increase of the octane number of the fuel [33]. Beyond the ratio of 30%, the RON increased up to 7.5%. The same results were reached by (*Najafi et al*) [34]. Note that ethanol has also environmental benefits by reducing engine emissions. Thus making the blends using ethanol DSB and dates mixture suitable for a spark-ignition gasoline engine. Whatever oxygenated boosting agents have been added to gasoline fuels, the octane numbers measured were above 96, so that, more power and acceleration can be produced by an engine. It has been noticed due to their oxygen content they are added also to reduce the carbon monoxide and hydrocarbon in the emission [35].

Sulfur content

Sulfur compounds in petroleum products may be present in different forms such as dissolved free sulfur, hydrogen sulfide, thiophenes, mercaptans, sulfides, sulfoxides,…etc. Their presence was undesirable for the reasons of corrosion, catalyst poisoning, bad odor, poor burning, and air pollution [36]. Most of the sulfur compounds were concentrated in heavier fractions. It characterizes the corrosiveness of the fuels, therefore, the environmental requirements for low sulfur levels in fuels are steadily rising.

The sulfur content for different blends is given in Fig. 9. It can be seen that the sulfur content of gasoline fuels does not exceed 0.014% by weight. The Sulfur content of gasoline fuels with oxygenates additives blends fell in the range of 0.0137 to 0.012 wt% while it was higher when adding ETBE, it ranges between 0.027 and 0.018 wt%. These results were in agreement with those found by *(Al-Baghdadi; Hsieh et al*) [33, 37], they found that the addition of ethanol led to reducing the gasoline sulfur content in the blends. Blends with less than 1 wt % Sulphur are referred to as low sulfur or sweet, and those with more than 1wt% sulfur are referred to as high sulfur or sour [38]**.** In this study, blends using ETBE/ ethanol in super-premium gasoline fuels were considered sweet fuels. Low-sulfur gasoline allows an immediate reduction in emissions from current engines and it is necessary for enabling the use of improved catalytic converters. Blends tested in this study have high octane rating and low sulfur content, i.e. they are not corrosive and can help to reduce air pollution.

CONCLUSIONS

The main conclusion of this experimental work is that the date stems by-products with fruit mixtures as an attractive way to produce ethanol. This residue is considered an interesting energy material due to its availability and abundance in many aride regions which could reveal better financial management. The effect of natural sugars from some fruits (Figs, sugar beets, dates) has been assessed through the conversion of sugars from date stems into bioethanol by using the Strain of VdH2 of *Saccharomyces cerevisiae*. The highest ethanol concentration was reached for DSB+20% dates, it was about 19.38 g/L after 72h fermentation process. The level of bioethanol production can be ascribed to the initial amount and uptake of sugars by the yeast growth. A good agreement was observed between the modified Gompertz model and experimental data, giving a good fit with R^2> 0.94, and the RMSE value fell within the range of 0.081–0.097 in all cases.

Besides, the addition of 20% ethanol or ethyl tertiary-butyl ether to super-premium gasoline increased the RON from 96 up to 99.3 and from 96 up to 99.8 in the case of DSB+20% dates or ETBE, respectively leading to the Sulphur content reduction in the range of 0.0137 -0.012 wt%.

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