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Optimizing Bioethanol Production for High Octane Bioethanol-Gasoline Blended Fuel through Fermentation

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Abstract: The present study is to investigate the potential bioethanol production from seasonal fruit wastes as a possible substrate via biochemical fermentation. It is worth mentioning that the waste feedstock was subjected to a pretreatment process before the fermentation process. The fermentation was carried out using cost-effective dry yeast such as Saccharomyces cerevisiae for 5 to 8 days. The main target of this research is to determine bioethanol percentage from fruit wastes that produced through optimization of the bioconversion process. Besides, the selected fruit wastes were evaluated and analyzed for variations in key parameters, which include sugar content, pH value, temperature, alcohol concentrations, and yield during yeast fermentation reaction at 32 °C for the production of alcohol. The present work exhibits a promising approach for bioethanol production on a large scale from inexpensive organic wastes and yeast. Moreover, the bioethanol obtained was blended with pure gasoline to produce ethanol-gasoline blended fuel in various proportions of 0%, 5%, 10%, and 15%. The resulting alternative fuel characteristics were assessed experimentally using American Society for Testing and Materials (ASTM) standards. The bioethanol-gasoline blends including Ried vapor pressure (RVP), density, and Research Octane Number (RON) was determined according to ASTM standard methods. Overall, the results showed that the RON of gasoline was enhanced remarkably with the increase in ethanol ratio.

Keywords: Bioethanol; fermentation; Saccharomyces cerevisiae; Research octane number (RON).

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1. INTRODUCTION

Nowadays, the growth of the power crisis increases the universal concern as a result of the dependence on traditional fossil fuel, which is being depleted gradually to meet the continuously growing demands in the energy market (1-5). Globally, biofuel is considered an alternative energy source because of its economic and environmental considerations. This type of fuel poses no threat to environmental life, thus helping to effectively decrease greenhouse gas emissions and security of energy supply, which leads to their increasing use (6). It is well known that bioethanol is type of biofuel, which produced through fermentation of saccharide and starchy sources that obtained from plants or algae such as corn grains, sugar-cane, and lignocellulosic biomass, etc. (7). It is an appropriate alternative fuel relative to fossil fuels. Furthermore, it is not a petroleum distillate that can be easily produced via agricultural feedstock or fruit waste (8,9). From another perspective, transforming waste biomass to biofuel and in turn, decreasing the enormous usage of conventional fuels is considered a great achievement in the global energy market. Moreover, the consumer's food chain will not be affected by using various sources of agricultural waste products as the feedstock of bioethanol production. On the other hand, the key fermenting agent such as yeast has contributed to reduce the cost of conversion biomass to bioethanol, it is preferable to use a cheap yeast that is available in local markets, and it is more economical in comparison with other fermentation agents (10). High volume and variety of organic wastes in main central marketplaces are being generated daily, especially with growing up the consumption of local fruits and producing more waste fruits (11). Approximately 50% of fruits and vegetables produced worldwide become by-products (as solid organic waste) during production processing as estimated in recent available data (12), hence fruit and vegetable biomass is a challenge to the environment all over the world and therefore there is a need to be recycled. This organic waste is a resource of potential energy and significant renewable fuel (13), thus biomass waste will help to produce the cheapest fuel as a result of the conversion of biomass to bioethanol (12). Bioethanol has been employed as an alternative fuel for internal combustion engines due to its renewable nature (14), which is considered the most promising and environmentally friendly renewable liquid fuel over conventional fossil fuel (15). It was clear that bioethanol leads to reducing the net emissions of CO_2. Furthermore, it can boost the octane rating of gasoline (when blended with it by a certain percentage) compared to standard gasoline (14, 16). The gasoline/ethanol mixture can be one of the alternative fuels since ethanol has high octane number compared to pure gasoline. This blended fuel can be utilized in an internal combustion engine without any additives. However, ethanol-gasoline blend fuel will minimize the heating value of the blended fuel as a result of low heating value of ethanol (17).

This study aimed to convert the organic fruit wastes as cheap sources to bioethanol via a fermentation process, followed by the bioethanol being purified using a distillation process to obtain high-purity ethanol for fuel purposes. Bioethanol obtained was blended with gasoline by different ratios to enhance the quality of standard gasoline for a spark-ignition engine. Bio ethanol-gasoline blend was analyzed and tested by various techniques to determine the influence of ethanol on the performance of the fuel.

2. EXPERIMENTAL SECTION

2.1. Materials

All the chemicals and reagents were purchased from Sigma-Aldrich, and Fisher Ltd and used as received without further purification such as 3, 5-dinitro salicylic acid (DNSA), potassium permanganate sodium potassium tartrate, urea reagent and D(+) glucose. White sugar (source of sucrose) and Saccharomyces cerevisiae yeast were commercially available in the main markets.

2.2. Bioethanol Fermentation Procedure and Parameters

2.2.1. Collection and preparation of decaying fruits

The raw samples of overripe fruits like black grape, banana, and red apple were collected from the main local fruit markets, located in Mosul city in Iraq, and then waste fruit samples were packed in a sterilized plastic bag and stored at low temperature in a research laboratory of Chemistry department of Mosul University in a refrigerator until further usage. About 1 Kg of each selected waste fruit was surface sterilized by sodium chloride (NaCl) solution and then rinsed well with distilled water (D.W). The selected waste sample was crushed individually in an electric grinder until it became liquid juice. The extracted fruit juice was collected and transferred to a 1 L flask and then diluted using D.W to 1 L (mix 1). Upon completion, the mixture was heated up to 95°C for 2 hours. The fruit juice was cooled down at ambient temperature and then stored in the refrigerator for further usage.

2.2.2. Fermentation process:

The fermentation method, according to the procedure described in Rishabh and Raj (18), was adopted for bioethanol production with a slight modification. Dried yeast (S. cerevisiae (E 491)) (50 gm) was inoculated into 300 mL of D.W and placed in a 500 mL conical flask under stirring conditions. Followed by 7.5 gm of urea reagent and 235 gm of sucrose or normal white sugar were added to the yeast mixture (mix 2) and then left to stir for 15 minutes at 35–40 °C for activation. Upon completion, the activated yeast inoculum (mix 2) and extracted juice (mix 1) were immediately poured into a 5 L conical flask, followed by D.W was added to a final volume of 3 L. Lab-scale batch of anaerobic fermentation was carried out in a sealed glass vessel of incubator that designed to conduct fermentation reactions. During the fermentation process, the yeast converted the waste sugar source into bioethanol and carbon dioxide as released gas. In the dark, the fermentation of rotten fruit samples was allowed to take place for 5 to 7 days at 32± °C with an agitation speed of 180 rpm. When carbon dioxide stopped to flow into the gas trap, it is considered a good indication of the end of the fermentation reaction. It is worth mentioning that test samples were taken from fermented solution before and after the fermentation process to evaluate bioethanol production and sugar content in the substrate respectively. In addition, the pH value of each fruit waste was measured and recorded before and after fermentation. Upon completion of the fermentation, the next step run was taken out from the incubator. The raw bioethanol, which is obtained by fermentation of waste fruit, was purified and concentrated by using a distillation unit to maximize the alcohol percentage in the final product. At the beginning of the distillation process, simple distillation was performed to produce the distilled liquid (bioethanol) in the range of 45-55 % at 78 °C, but the bioethanol required further purification for fuel purposes. Consequently, the resulting bioethanol was subjected to further purification through fractional distillation at 78 °C, which concentrated to 95% in the second distillation. Furthermore, hydrated bioethanol (95%) was dried using a 3A° molecular sieve to afford anhydrous ethanol (99%) that measured by hydrometer (alcoholmeter). Bioethanol produced was analyzed and confirmed by using FTIR technique.

2.2.3. Estimation of reducing sugars in fermented solution by (DNSA) method:

The reduced sugar content in the fermented solution was measured using DNSA method, which described by Garriga et al (19) with some modifications. (DNSA) the reagent was prepared by dissolving 0.5 gm of (DNSA) in 100 mL of D.W and the solution was stirred at room temperature. Then an aqueous solution (2N) of NaOH (7.5 mL) was slowly added to the DNS solution and stirred at ambient temperature until obtaining a clear solution. A solution containing (15 gm of sodium potassium tartrate in 20 mL of D.W) was added to (DNSA) solution followed by the mixture was filtered by filter paper and diluted with (D.W) to 50 mL using a volume flask. The DNSA reagent solution was kept at a low temperature (below 5°C) in a dark glass bottle. Stock standard solution of glucose, at a concentration ranging from 100 µg/mL (0.1 mg/ mL) to 1000 µg/mL (1 mg/ mL), was prepared using dried test tubes, then standard solutions were diluted to 2 mL with D.W to each test tubes, then 1 mL of DNSA solution was added to each tube and mixed well then covered all tubes with peace of cotton to avoid the loss of liquid due to evaporation. The tubes were kept at 95 °C for 15 minutes in the water bath to develop the red-brown color. After cooling, 5 mL of D.W was added to each test tube to stabilize the color. Thereafter, absorbance was measured by a spectrophotometer (UV-Vis spectrometer-PG instrument limited- Model T92+) at 540 nm.

2.2.4. Determination Of pH:

pH value of the fermented solution was determined and recorded during the fermentation process using a digital pH meter (Eutech instruments- PC 700).

2.2.5. Determination Of Ethanol Concentration:

The collected distillates for each purification step were measured using a hydrometer tool; it was widely used to determine the concentration of alcohol. At 20 °C, a distillate being tested should be placed in a graduated cylinder then the hydrometer would be allowed to float on the distillate to measure and record the concentration of alcohol (%) as shown in Figure 1.

Figure 1: Determination of bioethanol Concentration using a gradual cylinder and hydrometer.

2.2.6. FTIR Spectroscopy:

Fourier Transform Infrared Spectrophotometer (FTIR) is a powerful technique that can be employed to identify some of the functional groups present in a solid, liquid, or gaseous sample. In our study, the functional groups of the bioethanol are analyzed by using FTIR (Bruker Alpha II-ATR, Germany). The absorption frequency spectra are recorded and plotted as transmittance vs wave number. The functional groups in bioethanol from different fruit wastes were confirmed and determined using FT-IR spectroscopy. In brief, bioethanol (1.0 µL) was placed on a fused KBr disc, which is mounted on the cell holder and fixed on the sample beam of IR spectrometer. The running was performed over a spectrum range of 400 to 4000 cm-1 and averaged 16 scans.

2.2.7 Bioethanol-Gasoline Blend Characteristic Tests

2.2.7.1. Sample preparation:

Bioethanol-gasoline blended samples were made by mixing normal gasoline with high-purity bioethanol (99%) in various blended rates (0%, 9%, 11%, 13%, and 15% vol/vol) as shown in Figure 2. The blending process was conducted in a glass bottle which tightly closed under the stirring condition at room temperature for 5 minutes. All tests of bioethanol-gasoline binary blends were carried out in the department of laboratory and quality control: at Baiji refinery in Iraq.

Figure 2: Collected bottles of bioethanol-gasoline blended samples.

2.2.7.2. Density Test:
The density of each tested sample was determined according to ASTM D4052 (20), using a digital density meter (Rudolph Research Analytical density meter-DDM 2911) as depicted in Figure 3. The fuel sample was injected in digital density meters to determine the density value at 15.5 °C.

Figure 3: Digital density meter apparatus.

2.2.7.3. Reid Vapour Pressure (RVP) Test:
The vapor pressure of pure gasoline and gasoline blends was measured according to the ASTM-D6378 standard (21), using a commercial RVP apparatus (Eralytics Eravap, Vapour Pressure Tester) as depicted in Figure 4. The method covers the use of automated RVP instruments to measure the vapor pressure exerted in a vacuum by hydrocarbon-oxygenate mixtures such as bioethanol-gasoline blended fuels.

Figure 4: Reid vapor pressure apparatus.

2.2.7.4. Research Octane Number (RON):
An octane number is considered one of the major characteristics of gasoline that must be measured accurately for motor fuels like gasoline. RON value of each bioethanol-gasoline blend was determined by a cooperative fuels research engine (Single-cylinder, four-stroke, and spark ignition engine) as shown in Figure 5, for determination of research octane number for base fuel and blended fuels. This method was carried out according to the standard method (ASTM-D2699) (22).

Figure 5: Single cylinder –test engine assembly for RON measurements.

3. RESULTS AND DISCUSSION

The anaerobic fermentation process of overripe fruits was carried out for 5-7 days at 32±2 °C, which produced bioethanol. Bioethanol yield was investigated at different types of fruit waste, black grape waste produced the highest amount of bioethanol (10.9%) with high purity (99%) as shown in Figure 6, whereas red apple ranked second, followed by banana produced the least amount of bioethanol at the same fermentation conditions. Hence, S. cerevisiae has achieved better in grape waste than the other fruits' wastes. A previous study showed that bioethanol yield from grapes, apples, and bananas was around 6.2%, 4.7%, and 5.4% respectively (23). In another study, bioethanol yields from grapes and apples were 5.2% and 4.5% respectively (18). Many studies have indicated that temperature also plays a crucial role in the fermentation process. Previous studies showed that bioethanol production enhanced with the increase in fermentation temperature that reached a maximum value at 35℃. However, further increasing or decreasing temperature leads to reducing the percentage of bioethanol production, this is probably due to the denaturing of the yeast cells used (24).

The maximum bioethanol productivity can be obtained depending on the amount of raw material, which means that the bioethanol concentration rises with the increase in sugar content in raw materials. In this study, concentration is the measure of alcoholic content that presented in the distilled liquid. Bioethanol concentration can be expressed in terms of percentage (%). The yield percentage of ethanol was determined in the black grape at 10.9%, and the apple at 8.5%, whereas it was 3.9% for the banana as listed in Table 1 and depicted in Figure 6. With the increase in sugar content in a substrate, ethanol production increased significantly. Comparative studies of bioethanol productivity from varied decaying fruits exhibited that grape waste has higher efficiency compared to other fruit wastes such as apples and bananas. The fermentation process of grape waste is cost-effective and does not produce any toxic by-products. Hence, it can be applied on large scale for industry.

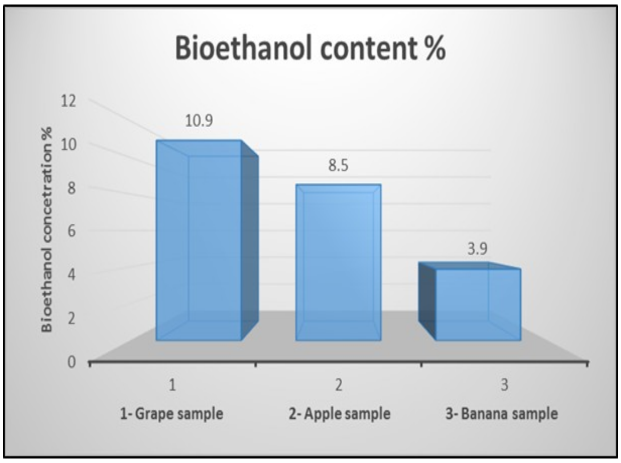

Figure 6: Graph showing a concentration obtained of bioethanol for each fruit waste during fermentation process.

Table 1: Various parameters obtained during fruit waste fermentation.

Sample	pH value before fermentation	pH value after fermentation	Bioethanol %	Fermentation period (days)
Black grape	4.5	4.9	10.9	7
Red apple	4.5	4.8	8.5	7
Banana	4.5	4.7	3.9	5

FTIR spectroscopy analysis identified the presence of methyl (-CH_3) stretch, hydroxyl (-OH) stretch, and –alkane (-CH_2) stretch in pure bioethanol. In the FTIR spectrum (Figure 7 A, B, and C) of each sample, a broad absorption band was found in a wave number range of 3317 cm^{-1} to 3331 cm^{-1} (slightly different in grape, apple, and banana), which corresponds to the OH stretching vibrations. Another peak is assigned at 2973 cm^{-1} as a sharp peak of stretching vibration due to the presence of the methyl group. Previous studies confirmed that wave numbers 2,900 and 3,300 cm^{-1} in FTIR graph of ethanol have been linked to CH and OH molecule groups, respectively (25). Notably, absorbance bands between 1045 cm^{-1} and 1380 cm^{-1} were observed due to stretching bands of the -CH_2 functional group (26).

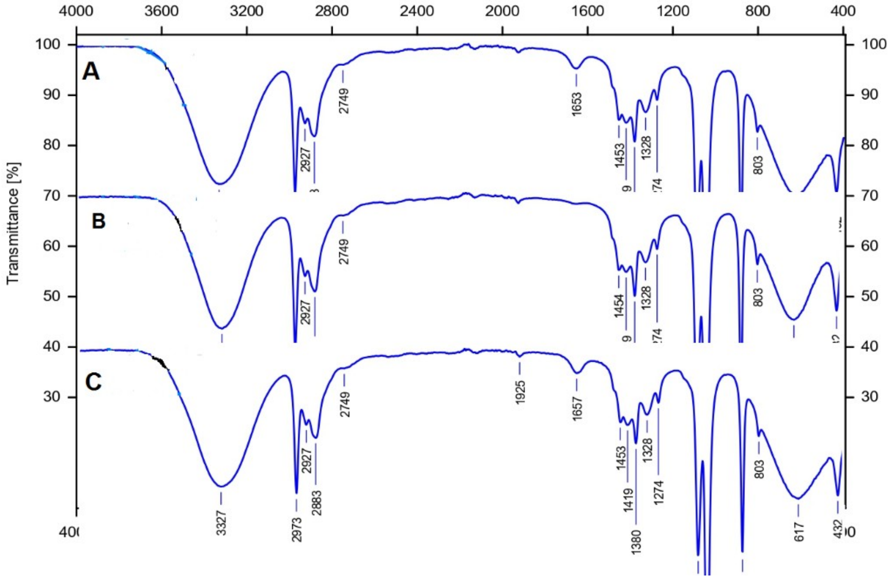

Figure 7: FTIR spectrum of distilled bioethanol, which produced from (A) black grape waste, (B) red apple waste, and (C) banana waste.

The pH parameter has a considerable influence on the alcoholic fermentation process. Based on this work, the initial pH of bioethanol obtained from the selected waste fruits was determined to be 4.5 as shown in Figure 8, while the final PH value of bioethanol obtained was determined in the grape at (4.9), apple (4.8), and banana (4.7). It was noted that the pH value was slightly increased after yeast fermentation; this can be due to the conversion of glucose to bioethanol. In terms of yeast activity, yeast can survive in acidic conditions that ranged from 4 to 6 (23). Another study indicated that the optimum pH value for yeast fermentation to generate bioethanol is 4.5 (27). Janani et al. revealed that a similar range of pH values in waste fruits was observed, the pH value of bioethanol produced from decaying fruits was estimated in the grape at 5.4, apple at 4.5, and banana at 5.1, these results agree with our results (23).

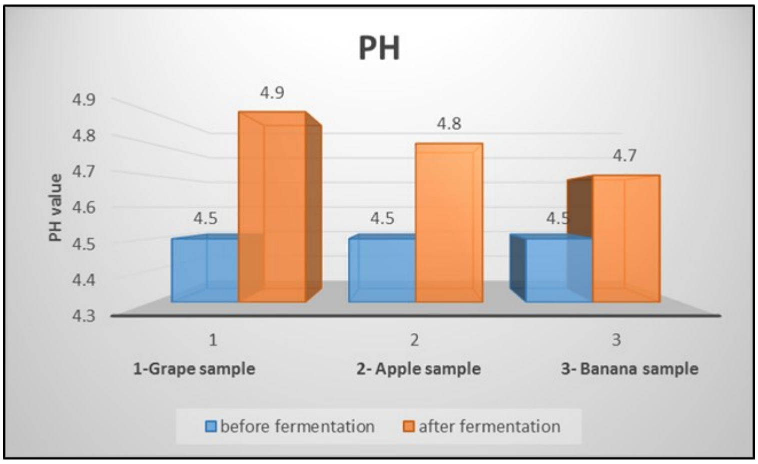

Figure 8: Graph showing a pH obtained of fermented solution for each fruit waste during fermentation process.

The standard curve of stock solution glucose was plotted to determine the reducing sugar and compared to each sample as depicted in Figure 9 a,b,c, and Table 2. The amount of sugar content was assessed using DNS reagent and it was found to drop down remarkably during the fermentation process. The sugar content of overripe fruit extracts was determined by comparing their absorbance taken from each sample to the standard curve of reducing sugar to calculate the sugar content at A 540. Among the three decaying fruit extracts used for the analysis of reducing sugar content, glucose content in grape solution extract dropped from 43 to 23 (mg/mL) after 7 days of fermentation, whereas glucose level in apple started from 43 (mg/mL) at the beginning of fermentation to 14 (mg/mL) at 7 days. A similar trend was observed in bananas, glucose level in banana waste declined from 18 (mg/mL) to 0.59 (mg/mL) after 5 days of fermentation.

Table 2: Estimation of reducing sugar via DNS standard method.

The volume of standard .glucose solution (mL)	The volume of D.W in mL	Amount of glucose in µg/mL	The volume of DNSA reagent in mL		dilution with D.W in mL	Absorbance at 540 nm
0.0	2	0	1.0 mL	Keep the test tubes in boiling water bath for 10 min	5.0 mL	0.000
0.1	1.9	100				0.010
0.2	1.8	200				0.049
0.4	1.6	400				0.139
0.6	1.4	600				0.267
0.8	1.2	800				0.348
1.0	1.0	1000				0.458

The reducing sugar concentration declined as the fermentation proceeds owing to the consumption of the sugar by Saccharomyces cerevisiae cells to produce bioethanol and carbon dioxide. It is worth mentioning that the initial glucose amount in extracted juice of the grape was diluted to 1:45 with D.W to obtain the absorbance into the standard curve, while the final sugar content of the grape waste extract was diluted to 1:25 with D.W. Other waste fruit extracts (apple and banana) were also diluted with D.W before and after the bioconversion process to measure the reducing sugar values as shown in Table 3.

Table 3: Estimation of sugar content in rotten fruit extracts before and after the fermentation process.

The volume of rotten fruit sample (mL)	The volume of D.W (mL)	The volume of DNSA reagent in mL		Dilution with D.W (mL)	Absorbance at 540 nm	Dilution (D) (mL)	Sugar content (mg/mL)
Grape-(b) (1.0 mL)	1.0	1.0 mL	Keep the test tubes in boiling water bath for 10 min	5.0 mL	0.439	45	43
Grape-(a) (1.0 mL)	1.0				0.417	25	23
Apple- (b) (1.0 mL)	1.0				0.436	45	43
Apple- (a) (1.0 mL)	1.0				0.450	15	14
Banana- (b) (1.0 mL)	1.0				0.420	20	18
Banana-(a) (1.0 mL)	1.0				0.249		0.59

(b): before fermentation process, (a): after fermentation processs, and (D): dilution factor.

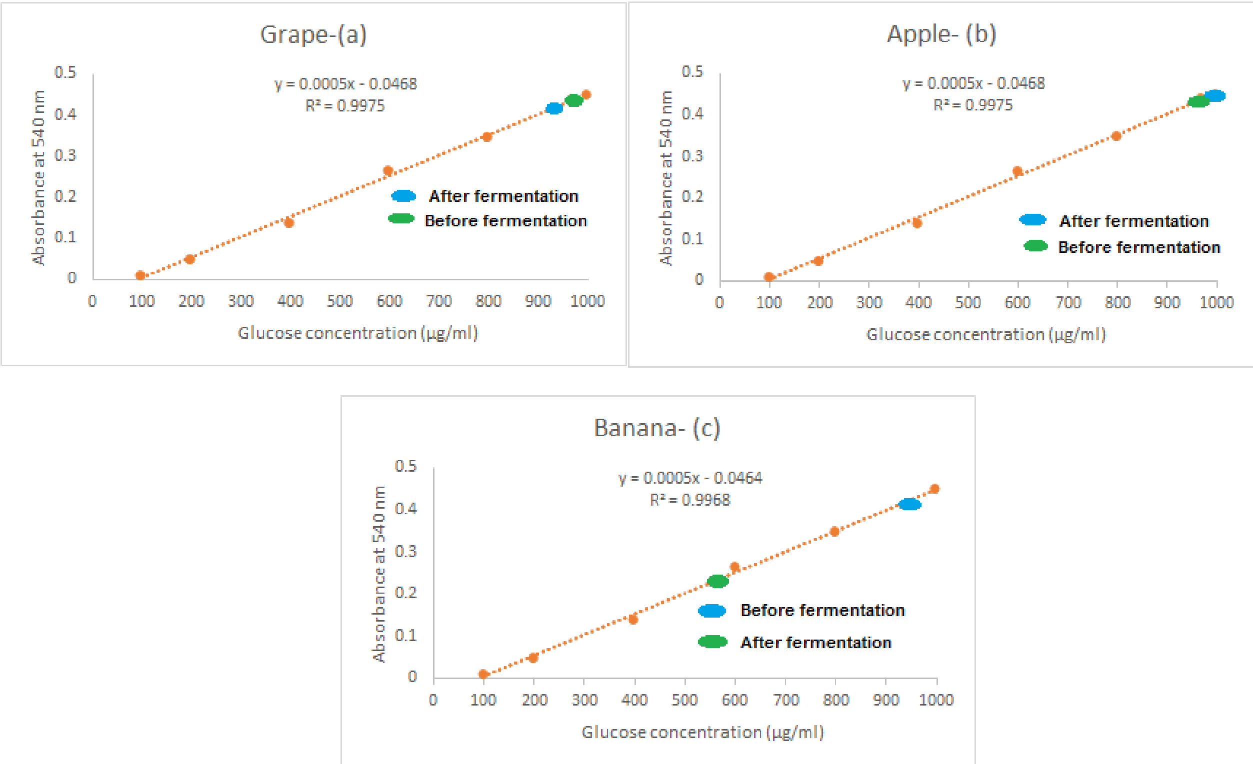

Figure 9: Estimation of sugar content in decaying fruit extracts before and after fermentation: (a) Grape waste. (b) Apple waste, (c) Banana waste.

The present work also focused on conducting tests toward bioethanol-gasoline blend characteristics and performance in different bioethanol ratios to analyze the probability of these blends as an alternative fuel. Therefore, different blend rates of bioethanol–gasoline blended fuels (9%, 11%, 13%, and 15%) were prepared and then sent to the petroleum quality control laboratory at Baiji refinery for ASTM standard analysis. The main results obtained from the ASTM analysis including RVP, density, and RON have been summarized in Table 4, to show the effects of bioethanol addition (9%, 11%, 13%, and 15% by volume) to gasoline on its performance. The results showed the variations of density (g/cm^3), RVP (psia), and RON, which considered as a function of different blend rates of bioethanol–gasoline mixtures.

Table 4: Specifications of regular gasoline and bioethanol-gasoline blends.

Characteristics	Test Method (ASTM)	Base gasoline	Bioethanol ratio in the fuel 9%	11%	13%	15%
Density (g/cm^3 at 15.5 °C)	*ASTM-D4052*	0.7305	0.7353	0.7369	0.7377	0.7395
RVP (psia at 37 °C)	*ASTM-D6378*	10.40	10.33	10.20	10.18	10.00
RON	*ASTM-D2699*	82.5	87.5	87.9	89.0	89.0
Color	Yellow	yellow	yellow	yellow	yellow	Yellow

Figure 10 represents the density values (g/cm^3) of the base gasoline and gasoline blends with bioethanol at various rates. The graph indicated that density increased with increasing the bioethanol content in the gasoline blend. The result is quite common due to the density of bioethanol that higher than the base gasoline.

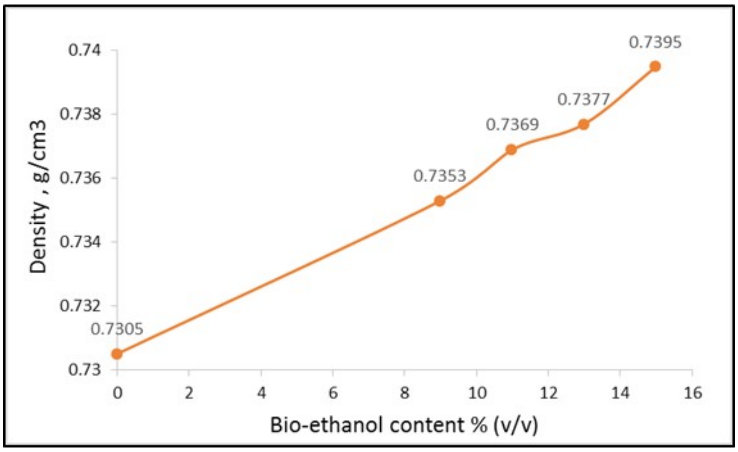

Figure 10: Graph showing density vs bioethanol content ratio to gasoline.

The behavior of bioethanol-gasoline mixtures was significantly different from conventional gasoline despite the fact that the RVP value of ethanol is much lower than that of base gasoline. The RVP value declined slightly when bioethanol was added to regular gasoline in various ratios as depicted in Figure 11. The decrease of RVP from fuel blends is caused by the little amount of water in the bioethanol-gasoline mixture by the increase of the volume of the alcohol mixture, and it may cause gasoline blend volatility change (water is more difficult to evaporate compared to gasoline and alcohol) (28).

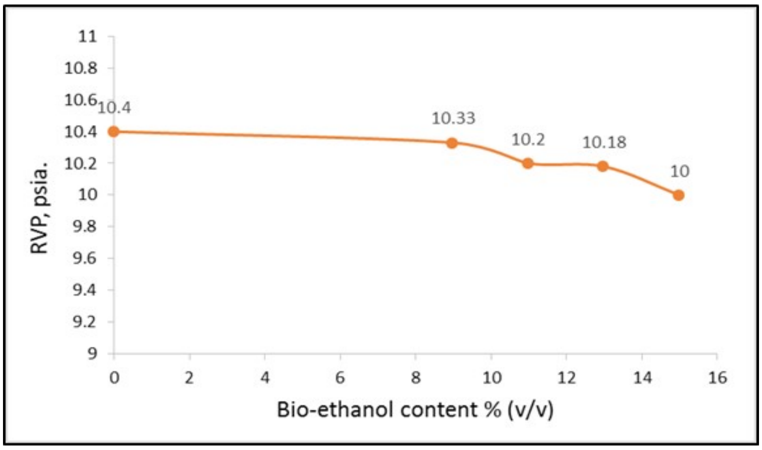

Figure 11: Graph showing RVP vs bioethanol content ratio to gasoline.

From the results of ASTM-D2699, RON of samples varies from 82.5 to 89 depending on the volume (%) of bioethanol added to the sample. The RON increases progressively with the increase of ethanol content as depicted in Figure 12, because of having a high RON value of pure ethanol at 105. It can be observed that the RON value jumped around 7 points when the bioethanol content exceeded 13% by volume, RON of blended fuel did not increase with increasing the bioethanol content to 15% by volume. Therefore, there is no need to increase ethanol content above 13% as it has a negative impact on RON parameter. The result obtained coincided with another study that investigated the impact of a gasoline-bioethanol mixture on the value of gasoline's octane number (16).

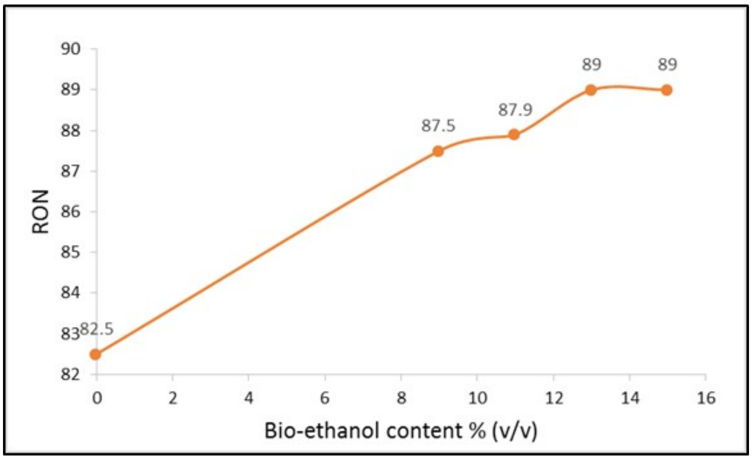

Figure 12: Graph showing RON vs bioethanol content ratio to gasoline.

4. CONCLUSION

In the present study, the results obtained revealed that different decaying fruits could serve as raw materials for bioethanol production via the bioconversion process. Moreover, bioethanol produced can be blended with pure gasoline in various proportions to be used as an alternative fuel to mitigate the demand for conventional fossil fuel resources. From this comparative study, the maximum bioethanol yield was obtained from grape waste (10.9%) followed by apple waste (8.5%) then banana waste (3.9) at 32 °C in acidic conditions (pH 4-5). The S. cerevisiae (yeast) was employed to convert saccharide wastes into bioethanol and carbon dioxide, and then a high concentration of bioethanol was obtained via simple distillation, fractional distillation, and dehydration respectively.

Bioethanol was mixed with conventional gasoline to produce blends that can be used as an alternative fuel for variable speed spark ignition up to 10 vol. % blends without engine modification. Analytical and experimental work on a single-cylinder engine was conducted to evaluate the effect of using bioethanol-gasoline blends instead of the base gasoline on the RON value, which considered a critical fuel property that plays a primary role in the design of the engine. It was clear that gasoline with ethanol content until 13% (v/v) can boost the RON value by 7 more points compared to base gasoline. These blends can be used by the vehicle engine smoothly without any engine modification. The comparative study showed that adding bioethanol to gasoline in different proportions has affected slightly on PVP and density values of blended fuels compared to regular gasoline. It can be concluded that gasoline with 13 %(v/v) bioethanol content can work well as a premium gasoline substitution.

5. CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

6. ACKNOWLEDGMENTS

We gratefully acknowledge Mosul Uniersity-Iraq for its support of this work. The present study was performed in the research center of the chemistry department at Education College for Pure Science. Furthermore, Gasoline blended samples were sent to the petroleum quality control laboratory: Baiji refinery in Iraq, to characterize and analyze their properties.

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