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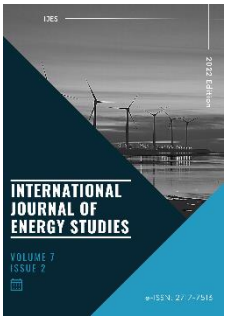

Research Article

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Production and determination of properties of ethanol from mango and orange peels

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Highlights

- Bioethanol has been produced from two samples of fruit wastes namely Mango and Orange peels.
- Sample A (mango peel) was identified as the feedstock that gives the highest yield of bioethanol.
- The properties of produced bioethanol were compared with standard ethanol.
- The properties of the bioethanol produced were analysed using the Duncan multiple range test (DMRT).

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ABSTRACT

The use of fossil fuels in IC engines in vehicles and industries has been identified as the leading cause of pollution, especially in urban areas where the traffic is usually heavy and industries are situated. In addition to air pollution which has its attendant health risks, the emission of greenhouse gases from the combustion of fossil fuels has been identified as one of the leading causes of climate change. The aforementioned reasons coupled with the fact that fossil fuels are exhaustible resources have necessitated the search for alternative eco-friendlier and sustainable fuels. The utilization of agricultural waste such as (mango and orange) peels to produce bioethanol proves to be a better alternative than the use of food crops. In this research, enzymatic scarification of yeast (Saccharomyces cerevisiae) was used to produce bioethanol from samples of mango and orange peels and their properties were compared with those of standard pure (98%) anhydrous ethanol. ASTM standards were used as comparative measures of the fuel properties that derived from bioethanol. It was found that the bioethanol concentration for the two samples were 19.98% for sample A and 19.17% for sample B and the results also show a good agreement as analysed by (ANOVA).

Keywords: Bioethanol, IC engines, fossil fuel, mango peels, orange peels

1. INTRODUCTION

Energy is one of the most important factors to global prosperity, the need to meet the insatiable global need for energy has seen a consistent increase in the demand for fossil fuels and consequently a consistent increase in the prices of petroleum products globally [1]. The high dependency and usage of fossil fuels are not without its attendant consequences on the environment and the health of individuals, these consequences which include but are not limited to air pollution in the form of smog, global warming and heat waves have led to the world seeking alternative energy sources that are environment and renewable. Amongst such alternatives are biofuels [2].

Bioethanol is the most used type of biofuel globally, its inherent features when compared to gasoline and other biofuels like high combustions products (oxygen content and octane number) which have endeared its usage as a choice biofuel which when fully deployed holds the promise of ensuring a free energy with non-pollutant to the environment [3]. Bioethanol can be derived using fermentation of micro-organisms of agricultural wastes like fruit peels, seeds and pulps. About 50% of these agricultural wastes are from fruit peels wastes which are inadvertently discarded as they are considered not to have any use [4]. Fruit peels represent an important source of sugar, which makes them an interesting choice for the production of chemicals such as ethanol [5]. Due to their abundance and renewability, these wastes such as (mango and orange) peels are being considered as raw materials for the production of competitive bioethanol in the open market [4]. Thus, production of bioethanol from these fruit residues could be major alternative for the disposal of these residues due to their carbohydrate content, which is similar to other bio feedstocks as observed by researchers [7].

In Nigeria and other developing countries where mangoes and oranges are being grown, there is a high rate of wastage of the fruits and also their peels as these peels are considered waste especially during their peak harvest season as they can be seen left to litter the surrounding and become potential sources of disease outbreaks and economic losses to farmers both in the farm and in the markets, therefore the use of this waste for bioethanol production aims to compensate for the economic loss of the farmers and free the environment from the negative effects [6]. Bioethanol production from these waste fruits peels which can be used as a supplement or in complement to fossil fuels is akin to killing two beds with one stone. Despite the focus by researchers to study the production of bioethanol from orange and mango pulps [7-10], little research exists on the

possibility for bioethanol production from mango and orange peels which usually go to waste and can become valuable low-cost resources for the production of bioethanol because it contains high rate of starch.

Acid hydrolysis of starch to glucose is one of the most trending in the world of researchers, and yet there is a little or no attention has been given by the researchers to using mango and orange peels as a substrate for the production of bioethanol. Usually, biomass produces from carbohydrate such as starch depends solely on the feedstock and the methods for production of bioethanol viz, pre-treatment, hydrolysis, fermentation, distillation, and dehydration [11]. It was reported that fuel that is generated from bioethanol has more oxygen molecules which contribute to faster flame speed that increase combustion initiation, stability and improves efficiency for spark-ignition (SI) engines [3, 12].

This research aims to use different fruit wastes (mango and orange peels) for the production of bioethanol which serves as a fuel for SI engines due to it is lignocellulosic biomass by fermentation process using rotten potatoes and tomatoes as vegetable medium i.e. potatoes dextrose agar (pda) to generate the cultured enzymes (saccharomyces cerevisiae) for the fermentation process and determine the properties of the ethanol produced. Two species from fruits peels were selected in this study namely: mango and orange peels due to their good amount of reducing sugar and low nutrient availability in order to get the best properties of bioethanol when compared with standard pure (98%) anhydrous ethanol.

2. MATERIALS AND METHOD

The basic biology behind bioethanol production is the action of microorganisms in the form of yeast anaerobic on a sugar-containing solution and its subsequent conversion into alcohol. The reaction involved can be represented by the following equations.

$$Sugar + Yeast \rightarrow Ethanol + Carbon\ dioxide \quad (1)$$

$$C_6H_{12}O_6 + Yeast \rightarrow 2C_2H_5OH + 2CO_2 \quad (2)$$

Starch is first converted to glucose which is then fermented into bioethanol using the equipment (see Subsection 2.1.2) and reagents mentioned in the chemical equations show these transformations as follows:

$$Starch + Water \rightarrow Glucose \quad (3)$$

$$C_6H_{10}O_5 + H_2O \rightarrow C_6H_{12}O_6 \quad (4)$$

2.1. Materials

Two samples A and B from fruit wastes, namely, Mango Peels and Orange Peels respectively were used as raw materials for this research work. The fruit wastes were collected from Gamborou market which is the major fruit market in the city of Maiduguri which is the capital city of Borno state, Nigeria. Waste potatoes and tomatoes for preparing vegetable media were also collected at the same market.

The following are the apparatus used in conducting the experiment viz: conical flasks, test tubes, spatula, pipette, thermometer, thermostat, industrial oven, autoclave machine, refractometer, pycnometer, U-tubes capillary viscometer, distillation apparatus, flash point apparatus, pH paper and dry active yeast. Wheresas, the reagents are Sodium chloride (NaCl), magnesium chloride ($MgCl_2$), sodium nitrate ($NaNO_3$), and potassium chloride (KCl) as mineral salt medium (MSM), lactic acid ($C_3H_6O_3$), lactic phenol and dextrose, sodium hydroxide (NaOH), Hydrogen sulphate acid (H_2SO_4), ethanol, de-ionized water, Cultured enzymes as yeast (Saccharomyces cerevisiae) and buffer solution.

2.2. Methods

2.2.1. Experimental procedure - preparation and sterilization of growth media

All the glasses were put in a container with tap water and liquid wash was added and left to stay for 10-15 minutes and then washed with clean water to remove all stains and impurities. The items were then rinsed with distilled water, and sterilized with hot air in an oven for 30 minutes at a temperature of 180ºC. Two hundred (200) grams of potatoes were peeled, shed and boiled in a conical flask for 20 minutes, the content of the conical flask was then filtered and the filtrate was mixed with dextrose. Another conical flask was boiled with water inside it and nutrient agar was poured little by little and stirred to avoid the formation of lumps. The dextrose with the filtrate was

mixed with agar solution (as growth media). When a complete solution of potatoes dextrose agar (PDA) was achieved the conical flask was corked with a large amount of non-absorbent wool to prevent contamination and then covered with aluminium foil. The flask was sterilized in a clinical autoclave for 20 minutes at a maximum temperature of 120°C and operating pressure of 15 Psi [7]. The cotton plug was wrapped with aluminium foil to avoid possible moistening of the plugs by condensing steam. On attaining the required sterilization period (20 mins) the clinical autoclave was switched off and cooled to room temperature. The sterilized and cooled medium was kept in a refrigerator.

2.2.2. Processes for the production of bioethanol

Bioethanol can be obtained from either carbohydrate-based crops such as sugarcane, sugar beet, corn and wheat, or from lignocellulosic biomass obtained from agro wastes/residues. There are different methods of producing bioethanol, according to the U.S. Department of Energy [13], the two main methods for bioethanol production are hydrolysis of biomass by using enzymes and fermentation to convert sugars to ethanol.

The fermentation process was adopted for the production of bioethanol using enzymatic scarification of the yeast (Saccharomyces cerevisiae) for this research. The equation for the fermentation reaction, is shown in Equation (5) while the bioethanol production process is represented by a flowchart as shown in Figure (1).

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (5)$$

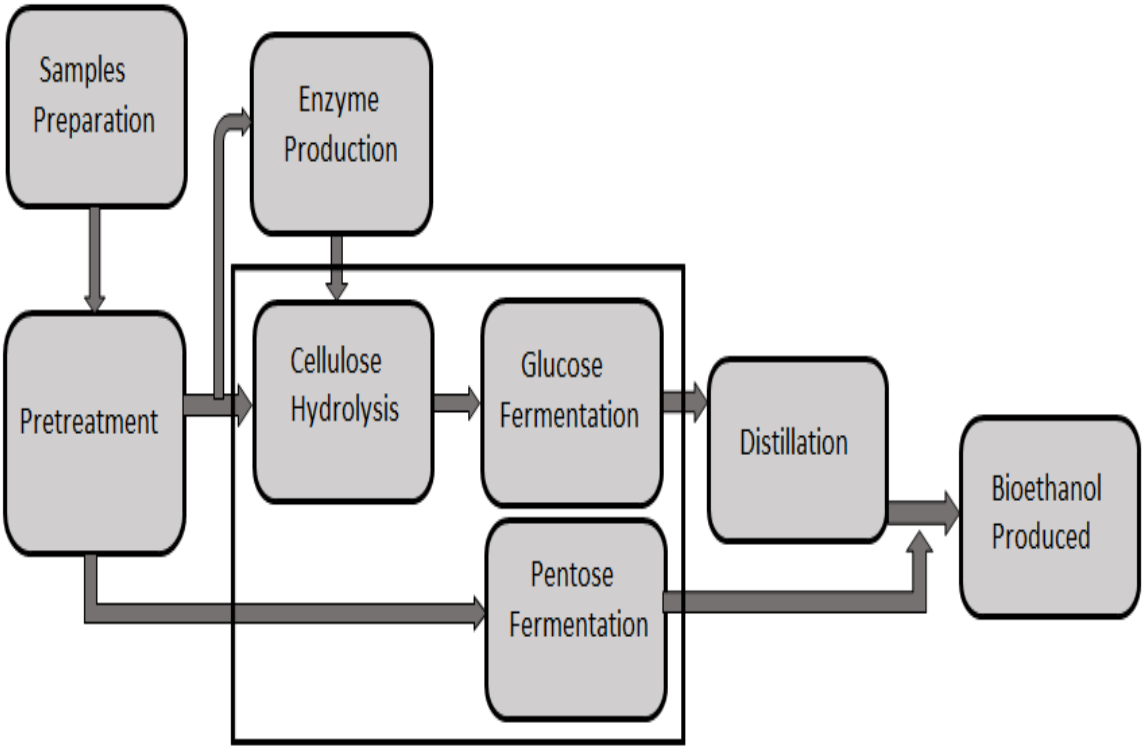

Figure 1. Bioethanol Production Process Diagram

a. **Sample collection and preparation:** Waste fruits samples of equal weights (100g) of mango and (100g) of orange as well as rotten potatoes and tomatoes as vegetable medium i.e. potatoes dextrose agar (PDA) were all collected from different locations in Gamborou market. The waste fruits were peeled, sliced into pieces and allowed to dry in a shade for a period of 120 hours. In order to make dried peels into powder, the electric grinder is used and poured in a conical flask at room temperature. The powder obtained was then weighed with the electronic weighing machine into two different samples A and B of equal weight i.e (100g) of mango and (100g) of orange respectively, they were then stored in airtight plastic containers. 600 ml of mineral salt medium (MSM) was poured into each sample, the MSM and the powder were thoroughly mixed and then the container was tightly closed for fermentation to take place.

b. **Pre-treatment:** This process is done to convert the cellulose for hydrolysis into fuels. The physical and chemical structure of the lignocellulosic biomass are changed during pre-treatment process and improve hydrolysis rates [14]. The peels were collected in a separate container and the hemicellulose fraction of the biomass was then splitted into simple sugars. The dilute hydrogen sulphute acid (H_2SO_4) is mixed with the hemicellulose fraction of the

biomass and this process is called Hydrolysis. The reaction obtained from hydrolysis process is the breaks down of the complex sugar chains (hemicellulose) and releasing simple sugars. These complex sugars are then changed into a mixture of soluble carbon sugars, mannose and galactose. In this step, a small part of the cellulose is also converted to glucose.

c. Production of enzyme: In order to hydrolyze the cellulosic portion of the biomass, the cellulosic enzymes grown-up. For this research, the enzyme was cultured using rotten potatoes and tomatoes as vegetable medium i.e. potatoes dextrose agar (PDA) to obtain the cultured enzyme (saccharomyces cerevisiae). The enzymes can be puchased from companies as an alternative method.

d. Cellulose hydrolysis: The purpose of this step is to hydrolyze the remaining cellulose into glucose. The cellulose enzymes are splitted into sugar chains which released glucose in the reaction for the enzymatic hydrolysis.

e. Fermentation: The glucose are converted into ethanol through microorganisms as a factor in fermentation. These microorganisms plays vital role in overcoming problems relate to lignocellulosic hydrolyzates occurs in this process. In this research, the concentration of ethanol is reduced by adjusting pH value of the samples to be above 5.0. This is done by adding 1M of Sodium Hydroxide (NaOH) into the samples [15]. These processes were carried out in small transparent gallons, each gallon containing hydrolysed sample mixed with 6.5g of the yeast and then the solution was shaked until required solution is obtained. These solutions were kept for about 100 hours to enable the fermentation process to be complete.

f. Distillation: This process was carried out in order to extract the bioethanol. The process was done using a set of distillation apparatus that is set at a temperature, pressure and speed of 55^{o}C, 0.175 bar and 70 rpm respectively. To eliminate the water molecules in the bioethanol, the dehydration process is done using anhydrous sodium sulphate (Na_2SO_4).

g. Ethanol recovery: This step is done to separate the ethanol from fermentation products by admitting anhydrous sodium sulphate (Na_2SO_4) to eliminate the water molecules from the bioethanol. Figure 2 indicates the steps to be adopted in the bioethanol production from fruit peels (mango and orange).

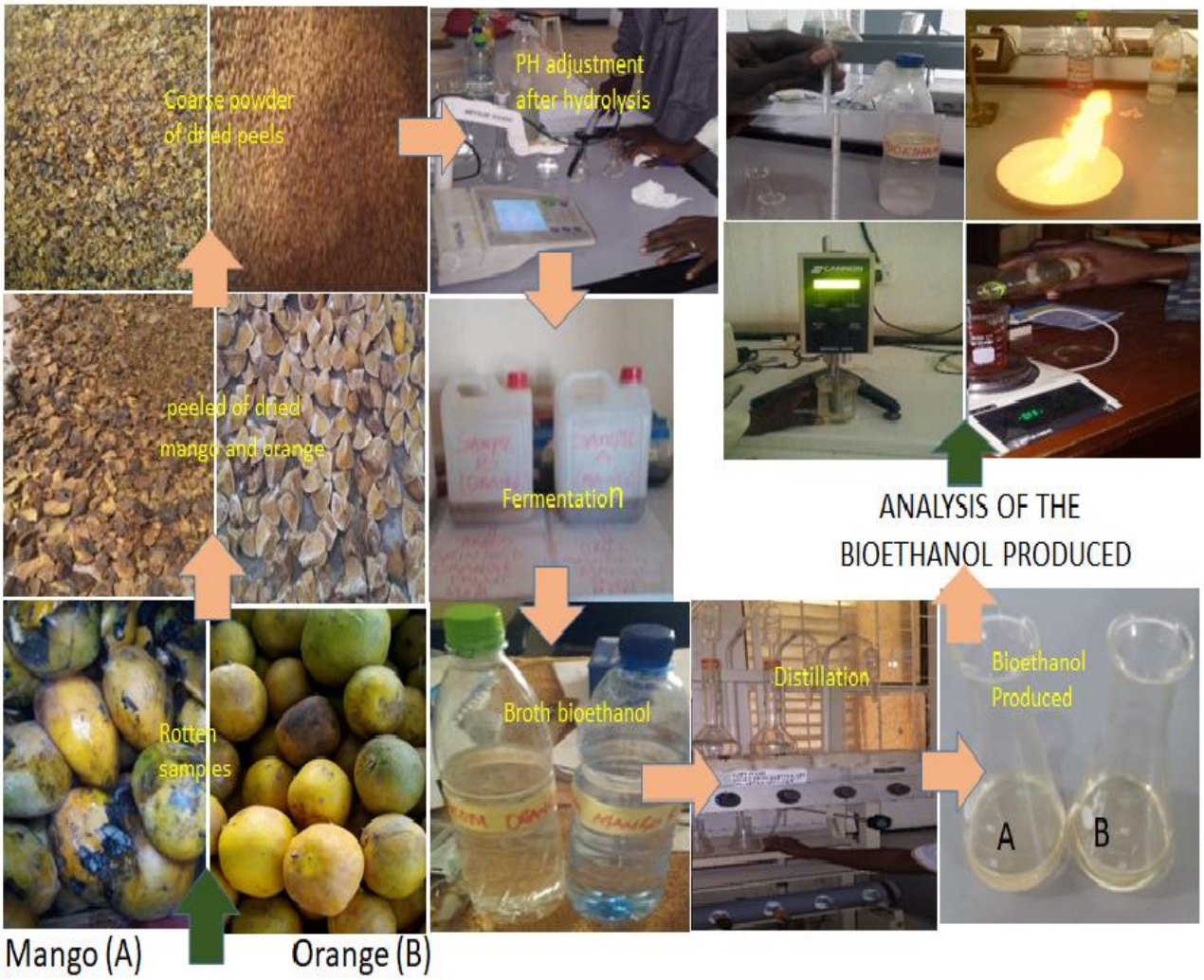

Figure 2. Processes involved in bioethanol production from fruits wastes (Mango and Orange Peels)

2.3. Properties of Bioethanol as a Fuel

The properties of bioethanol from fruit wastes were measured based on the ASTM standards. The experimental properties of bioethanol obtained from fruit wastes were compared with the ethanol ASTM D4806.

2.3.1. Ethanol recovery

The refractive index method was used for bioethanol concentration as described by [16]. The refractometer was used to obtained the refractive index and the corresponding values of bioethanol concentrations from the calibration curve, while the percentage yield of each sample was determined by the formula [17]:

$$Yield\ point\ (\%) = \left(\frac{Sample\ Weight}{Mass\ of\ Samle\ Distilate}\right) \times 100 \quad (6)$$

2.3.2. Flash point

50 ml of the sample was poured into penskymartens apparatus and it is then switched on. The samples were stired with the aid of stirrer in order to obtain steady temperature. When the blue flame of apparatus was obtained, the flash points of the samples were recorded. These procedures were concorded by [17].

2.3.3. pH test

The pH meter was inserted into the samples and recorded the pH values of the samples after standardizing the meter by placing it into a buffer solution.

2.3.4. Density and specific gravity test

Pycnometer was filled up with the samples and it is weight was recorded and the weight of the apparatus was also recorded when its empty. The density of the sample can be obtained using the equation (7) given below:

$$\text{Density } (\rho) = \frac{Mass}{Volume}\ \left(^{g}/_{ml}\right) \text{ or Density } (\rho) = \frac{M_2 - M_o}{M_1 - M_o} \quad (7)$$

Where:

$M_2 =$ mass of empty bottle in (g), $M_1 =$ mas of empty bottle $+$ water in (g) and

$M_o =$ mass of the substance in (g)

Similarly, the weight of the Pycnometer was recorded when filled with distilled water. The equation (8) given below is used to obtain the specific gravity (spg):

$$\text{Specific gravity (spg)} = \frac{\text{density of bioethanol}}{\text{density of water}} \quad (8)$$

2.3.5. Viscosity test

A viscometer with A-arm and B-arm capillary tubes was used to measure the viscosity of the samples. 50 ml of the sample was poured into the apparatus through the orifice to the marked point of the A-arm tube and a sucker was used in the B-arm tube to lower the sample to the marked

point. The time-taken for the sample to flow through the tubes to the mark under the B-arm tube was recorded using a stopwatch. To convert the viscosity into centistokes, it is recommendate to use viscosity calibration curve [18].

2.3.6. Boiling point

The boiling point of the samples were determined by noting the reading of the inserted thermometer through the cork covering the conical flask containing the sample being heated when it is boiling.

3. RESULTS AND DISCUSSIONS

Table 1 presented the pH values of the samples for the period of fermentation. It can be seen that the longer the samples stayed in the fermentation chamber the more the pH increases – the more the samples lose their acidity.

Table 1. pH values obtained from each sample during the first week of fermentation

Time (Hours)	24	48	72	96	120	144	168
Sample A (Mango)	5	5.26	5.45	5.84	6.50	6.85	6.72
Sample B (Orange)	5.16	5.18	5.60	5.64	6.23	6.60	6.81

The result in Table 2 shows that glucose concentration and absorbance from Sample A increase more rapidly than Sample B sample at the first week of fermentation, this is because sample A has lower acid content that requires less time to digest and ferment subsequently as compared to sample B with higher acid content and requires more time to digest before fermentation.

Table 2. Glucose test results obtained from each sample during first week of fermentation at intervals of two days.

Samples	Time (hrs.)	Quantity (g)	Absorbance	Conc. Mol/L	Conc. %
A	48	100	1.983	1.991×10^{-3}	0.199
B	96	100	1.944	1.951×10^{-3}	0.195

The result of Table 3 shows that sample A has the least moisture content (in percentage) but the highest percentage of ethanol while sample B has the highest moisture content (%) with the least percentage of ethanol. This can be attributed to the low acidity of the sample compared to sample B which has the high citric acid content. The concentration of bioethanol in sample A can be due to the effective activity of the saccharomyces cerevisiae as enzymes for the production of ethanol which is similar to the work of [19].

Table 3. Percentage of ethanol and moisture content of samples at the first stage of distillation

Sample	Mass (g)	Vol. of ethanol-water mixture (ml)	Moisture content (%)	Bioethanol (%)
A	100	590	23.73	76.27
B	100	521.6	29.00	71

The result presented in Tables 4 and 5 represents the density of ethanol at the first and second stages of distillation respectively. It can be seen from Table 4 that sample A has the least density of 849 kg/m^3 with a volume of 5.9×10^{-4} m^3 while sample B has a density of 899 kg/m^3 with a volume of 5.8×10^{-4}. Similarly, in Table 5 the volume of each sample decreases and the density also decreases. This shows that sample A is less dense and has 793 kg/m^3 with 4.5×10^{-4} m^3 while sample B is denser and has 800 kg/m^3 with 3.7×10^{-4} m^3, this confirms that the density of sample B is a result of its mass and volume in both first and second distillation as clearly shown in both Tables [12].

Table 4. Density of ethanol-water mixture of each sample at first stage distillation

Sample	Mass (M_1) (kg)	Volume (V_1) (m^3)	Density (ρ_1) (kg/m^3)
A	0.5006	5.9×10^{-4}	849
B	0.5216	5.8×10^{-4}	899

Table 5. Density of the produced ethanol at the second stage of distillation

Sample	Mass (M_2) (kg)	Volume (V_2) (m^3)	Density (ρ_2) (kg/m^3)
A	0.357	4.5×10^{-4}	793
B	0.296	3.7×10^{-4}	800

The yielded result of the composition showed an increase in bioethanol production (Table 6) for sample A and a decrease in sample B. The fermentation of waste fruits by saccharomyces cerevisiae for ethanol production had resulted in a high percentage of bioethanol. For this research, the overall percentage of bioethanol produced did not exceed 19.98% for sample A and 19.17% for sample B. The yield which is generally low is due to the small amount of sample used.

Table 6. Percentage of ethanol (% ethanol) yield from each sample used

Sample	Quantity of sample used (g)	Mass (g)	Yield point (%)
A	100	500.6	19.98
B	100	521.6	19.17

The results tabulated in Table 7 presents the estimated mean properties of the Bioethanol investigations carried out with respect to waste fruits of mango and orange as the feedstock. The statistical analysis (ANOVA) using Duncan Multiple Range Test (DMRT) was also used to analysed the fuel properties of ethanol samples. It is obvious from the result presented in Table 8 that the observed differences were not significant, however, the mean treatment difference observed between Sample 1 and 3 and Sample 2 and 3 for the flash point were significant at 5% level. This occurs due to the high moisture content present in the samples when compared with the control sample (sample 3). This is responsible for the lower flash point of sample 3 compared to that of samples 1 and 2 as seen in Table 7.

Table 7. Estimated Mean of Ethanol Properties

Samples	Flash point	Boiling point	Viscosity	PH	Refractive index	Relative density	Specific gravity
1. Bioethanol from Mango	28.6	79.6	1.4	5.3	1.36	0.91	0.79
2. Bioethanol from Orange	30.2	81.3	1.8	4.8	1.35	0.97	0.80
3. Absolute Ethanol	12.2	78.3	1.09	5.2	1.35	0.78	0.79

Table 8. Mean Treatment Difference of Ethanol Properties

	Flash point	Boiling point	Viscosity	PH	Refractive index	Relative density	Specific gravity	Df
1 VS 2	-1.6	-1.7	0.4	0.5	0.01	-0.06	-0.01	1
1 VS 3	16.4	1.3	0.31	0.1	0.01	0.13	0	1
2 VS 3	18	3	0.71	-0.4	0	0.19	0.01	1

Where 1= Ethanol from Mango; 2= Ethanol from Orange; 3 = Absolute Ethanol

4. CONCLUSION

Bioethanol has been produced from two samples of waste fruits mango and orange peels respectively with equal proportion through a fermentation process. Sample A (mango peel) was identified as the feedstock that gives the highest yield of 19.98% of bioethanol while sample B (orange peel) yielded about 19.17% of bioethanol. The properties of produced bioethanol were compared with standard ethanol, the properties showed similarities within 5% (average error) when analysed using the Duncan multiple range test (DMRT) in design expert 7.0 statistical package, which was in good agreement with that of pure ethanol requiring 5-10% gasoline addition for de-nurturing before use. Furthermore, bioethanol produced at the first stage of distillation should be subjected to another distillation or separation method such as fractional distillation, membrane separation etc., this is to refine the process in order to separate ethanol from moisture contents for absolute combustibility. The study further shows that waste fruits (mango and orange) can be put to use by increasing the scale of use of these waste materials for bioethanol production which will minimise the economic loss that may be accrued to the waste fruits and by extension contributes immensely to carbon sequestration.

DECLARATION OF ETHICAL STANDARDS

The authors of the paper submitted declares that nothing which is necessary for achieving the paper requires ethical committee and/or legal-special permissions.

CONTRIBUTION OF THE AUTHORS

Anas Bala: Brought the Idea and Planned the method. Performed the experiments and analyse the results.

Jamilu Ya'u Muhammad: Conducted the literature review and found the research gap. Re-examined the spelling and grammar of the article.

Richard Balthia Mshelia: Performed the experiments and analyse the results. Re-examined the spelling and grammar of the article.

M. Adam: Wrote the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

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