

Bio-Chemical Characterization of Fruit Wastes as an Alternative Feedstock for Bio-Ethanol Production and Performance Analysis with a Diesel Engine System using Gasoline and Ethanol Blends

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Abstract: The bio-ethanol derived from sustainable waste material has become most important in recent times as the world researches to find an alternative energy as a convincing supplement for fossil fuels. In addition, the possible exploration of waste fruit biomass being generated ceaselessly at the local markets is not only emerging as viable and clean bio-fuel, it is also promoting the reduction of atmospheric pollution and global warming. The current investigation was undertaken to evaluate the sustainable and techno-economic feasibility of utilizing different categories of waste fruits for the production of bio-ethanol by employing a cost effective simple approach called yeast (*Saccharomyces cerevisiae*) fermentation technology. After performing the successful fermentation processes, the bioethanol production shown that, 730 µg/ml; 640 µg/ml; 510 µg/ml; 470 µg/ml; 460 µg/ml and 420 µg/ml of ethanol from 100 mL of waste fruit juices covering of Grape, Banana, Orange, Papaya and Sweet Lime and watermelon respectively. The ethanol production has been accomplished after distillation and maintaining a pH of 5.5 and a temperature of 30°C. Interestingly, the mixed waste fruit samples was showed substantially superior yield of bio ethanol (590 µg/ml) as compared to individual class of waste fruit samples where the physio-morphological analysis was very complex compared to mixed fruit approach. In all, the significant bio-ethanol recovery was noticed at mixed waste fruit samples (10.95%) under the yeast concentration of 2 g/L with the temperature of 30°C. whereas, the individual category of waste fruit samples *i.e.*, grape fruits (11.35%) followed by watermelon (11.10%), pineapple (9.75%) and mango (8.65%) respectively. In the methodical experimentation, the increased ethanol production with increase in fermentation time until five days of incubation was recorded, where the glucose and pH were reduced during the fermentation process. The produced ethanol displayed extreme purity using GC-MS technique in comparison with the standard. In the meantime, the analysis of elemental composition (Al, Cu, Pb, Ca and Mg) of the mixed waste fruit sample was substantial and the viscosity, specific gravity, flash point, fire point and acid values of bioethanol were found to be within the limit of American Society for Testing and Materials (ASTM) standard specifications with less hazardous elements. Further, in the engine interactions, the bio-ethanol blends of WFBE5, WFBE10 and WFBE20 revealed that, the TFC with BMEP followed by BSFC with BMEP, BTE with BMEP at an engine speed of 2000 rpm were found to be excellent. This may possibly be significant in their expressions and found to be superior by increasing the compression ratio which could be accommodated for bio-ethanol blends since ethanol has a higher octane number rather than gasoline. In addition, at the peak of BMEP, the effect of bio-ethanol content on the volumetric efficiency was found to be more prominent for the WFBE10 blend. Later, the equivalence ratio of WFBE0 (100% gasoline only) was placed within lean limits at the lowest BMEP. The increased BMEP has a tendency of the mixture attaining at stoichiometric strength on the peak of BMEP. Furthermore, the engine-fuel interaction relating to emission parameters like, hydrocarbon, NO, CO and CO₂ content were considerably lower in WFBE5 (5% bio-ethanol-with 95% gasoline), WFBE10 (10% bio-ethanol with 90% gasoline) and WFBE20 (20% bio-ethanol with 80% gasoline) than in WFBE0 (100% gasoline) having less fuel consumption. The generated results in due course confirmed that production of bio-ethanol can be achieved from waste fruits (both individually and mixed fruits) and possibly standardized as it is extremely sustainable and also renewable sources. The WFBE fuel can be used in the existing engine system in combination with pure petro-fuel by way of an eco-friendly approach with no release of toxic gases to the environment. Besides, the reduced fuel consumption can be monitored by using this most promising ethanol practice resulting from the waste fruit feedstock apart from environmental waste management.

Keywords: Bio-Ethanol production; Fermentation Technology; waste fruit samples; *Saccharomyces cerevisiae* (Yeast); Engine-fuel interaction & Performance analysis

1. Introduction

The requirement for energy is relentlessly mounting up, because of rapid increases in industrialization and automobiles. The basic

sources of this energy are petroleum, natural gas, coal, hydro, nuclear etc. and the increasing concern of fuels as well as the escalating social and industrial awareness leads to exploration for the clean renewable fuels (Mohr *et al*, 2015).^[1] The possible exploration of alternative, potential and eco-friendly fuel sources for the

Table 1. Recent investigations made on the production of Bio-ethanol from waste fruit samples

Feedstock	Objectives focused	Major findings	Ref.
Grapes, Sugarcane, Mosambi and Watermelon	To produce Ethanol from fruit wastes using <i>Saccharomyces cerevisiae</i>	Optimizing several factors that influence the process for bio-ethanol production such as temperature, pH and sugar concentration	Babu <i>et al</i> , 2014 ^[68]
Banana and Mango pulp and peels	To Evaluate chemical composition of fruit wastes (pulp and peels) of Banana and Mango to ascertain their potential application in bioethanol production.	Significant outcomes on fermentation of hydrolysates from the dilute acid pretreatment followed by enzymatic saccharification of mixed fruit pulps (banana and mango). The banana fruit peels were found to be best for higher ethanol production at optimized conditions.	Arumugam and Manikandan, 2011 ^[41]
Pineapple wastes	To Produce of bio-ethanol from pineapple wastes, especially the fruit peels	Samples pretreated with 0% NaOH were subjected to microbial hydrolysis which showed an increase in reducing sugar of the samples. At the end of the experiment, a bioethanol yield of 5.98 ± 1.01 g/L from pineapple fruit peel was successfully produced at 48 h of fermentation.	Casabar <i>et al</i> , 2019 ^[52]
Sugar juice, starchy crops, and lignocellulosic materials	To achieve Ethanol production from free sugar containing juices obtained from some energy crops such as sugarcane, sugar beet, and sweet sorghum using three types of fermentation process i.e., batch, fed-batch, and continuous.	The fermentation factors greatly influences the process and their optimization is the key point for efficient ethanol production from these feedstocks.	Hossain Zabed <i>et al</i> , 2014 ^[50]
Orange, Sweet lime, and Banana peels	To understand the potential application of <i>Saccharomyces boulardii</i> derived bio-ethanol from fruit wastes was explored using orange, sweet lime, and banana peels mediated by yeast fermentation.	The chemical nature of fruit waste-resulting bio-ethanol was compared with commercial ethanol using gas chromatographic analysis. Bio-ethanol ratios i.e., 0%, 4%, 8%, 12% by volume as part of fuel blends were subjected to performance testing using a single-cylinder, four-stroke spark ignition engine. The operational parameters like total fuel consumption, brake specific fuel consumption, brake thermal efficiency, volumetric efficiency, and equivalence ratios were found to be significant. The bio-ethanol yields increased up to 24 h of fermentation which confirms the vital role of increased pH of the biomass after 24 h up to 72 h. The fruit derived bioethanol showed ideal physico-chemical characteristics for use as automobile fuel	Pai A. <i>et al</i> , 2020 ^[40]
Fruit and Vegetable Waste	To accomplish sustainable Alcohol Production process from Fruit resources.	The results reveals that, after 7 days of fermentation, pineapple peels had the highest biomass yield, followed by banana peels, orange peels and pea peels.	Shilpa, <i>et al</i> , 2013 ^[9]
Fruit wastes samples	To develop easier techniques by using cheaper source for the production of Bio-ethanol from fruit wastes with <i>Saccharomyces cerevisiae</i> which should be practiced by common people	The rate of ethanol production through fermentation of fruit waste yield is optimal at pH 5.5, temperature 32°C, specific gravity 0.865, and conc. of about 6.21%. The waste materials after the fermentation can be used as a soil fertilizer.	Hari Shankar <i>et al</i> , 2014 ^[13]
Sugarcane juice	To Evaluate the mineral composition in juice of three sugarcane varieties	The content of some important minerals, such as Cu, Mg, Zn and P were mentionable that varied with sugarcane varieties	de Souza <i>et al</i> , 2015 ^[44]
	To study the effect of mineral content in juice on the fermentation efficiency of <i>S. cerevisiae</i> . To assess the effects of yeast cells adaptation to galactose on the final ethanol yield during fermentation by a thermo-tolerant yeast strain (<i>Pichiakudriavzevii kudriavzevii</i>), isolated from sugarcane juice through enrichment technique	Maximum amount of ethanol was obtained from the highest Mg containing juice. A 30% higher amount of ethanol was produced by the galactose adapted cells than that of non-adapted cells. Fermentation with galactose adapted cells at 40 °C produced an ethanol concentration of 71.9 g/L and Productivity of 4.0 g/L/h.	Dhaliwal <i>et al</i> , 2011 ^[45]
Sugar beet juice	To study the effects of supplementation of juice with mineral salts on ethanol yield	A high concentration of ethanol was produced by the supplemented juice that ranged between 85 g/L and 87 g/L	Kawa <i>et al</i> , 2013 ^[53]
Sweet sorghum	To evaluate of ethanol yield of five sweet sorghum varieties, namely Keller, BJ 248, SSV84, Wray and NSSH 104.	Ethanol concentration in the broth varied significantly among the varieties, where the Keller variety produced maximum amount of ethanol (9%, w/v)	Ratnavathi <i>et al</i> , 2010 ^[60]

Watermelon juice	To investigate on Water melon juice as sweet sorghum varieties namely, Keller, BJ 248, SSV84, Wray and NSSH 104. The feedstock was analyzed for its feasibility to produce juice to achieve the ethanol. The efficiency of fermentation was evaluated with the addition of different sources.	Watermelon juice that did not contain lycopene but contained free amino acids, were readily fermentable either as main feed-stock or as diluents, supplement and nitrogen source to granulated sugar or molasses. A minimum level of 15 $\mu\text{mole/ml}$ amino nitrogen was required in the juice to achieve maximum fermentation rates when it was employed as the sole nitrogen source for the fermentation.	Fish et al., 2009 ^[47]
Dates	To analyze the comparative approach of direct and soxhlet extraction of sugars from three date varieties i.e., Kunta, Eguoua and Bouhatem.	Although direct extraction method provided slightly lower concentration of sugars (104.6–214 g/L) than soxhlet extraction (115.1–225.8 g/L), it required significantly lower time and energy	Louhichi et al., 2013 ^[55]
Sugar beet molasses	To study the date varieties for their bio-ethanol prospective. Studying the effects of using diluted molasses broth on ethanol yield. Studying the effects of pH and yeast inoculums concentration on ethanol yield.	Ethanol produced by the varieties was very close, around 25% (v/v) Dilution of the molasses negatively correlated with ethanol yield and undiluted broth produced maximum amounts of ethanol. Low pH (4) and moderate inoculum size (5 g/L) produced maximum amounts of ethanol	Marx et al., 2016 ^[56]
Whey	To investigate bio-ethanol production from whey in a fermenter integrated with direct contact membrane distillation (MDBR) To appraise the concentrated whey and de-proteinized whey enriched with either sucrose or lactose were used as fermentation media	A continuous removal of ethanol and other volatile compounds from the broth by membrane distillation resulted in a high efficiency of sugar conversion into ethanol. Fermentation rate is slightly faster in the integrated bioreactor than in the traditional reactor. The efficiency of ethanol production by the de-proteinized whey enriched with sucrose was exceedingly close to the theoretical value.	Tomaszewska and Białończ 2016 ^[72]
Mahua flower (<i>Madhuca longifolia</i> L.)	To compare the efficiency and yield during the fermentation of Mahua flower juice by employing widely used microorganisms i.e., <i>S. cerevisiae</i> and <i>Z. mobilis</i> .	<i>S. cerevisiae</i> showed higher fermentation efficiency than <i>Z. mobilis</i> , with 21.2% higher ethanol yield, 5.27% higher productivity and 134% higher sugar conversion.	Behera et al., 2010 ^[17]

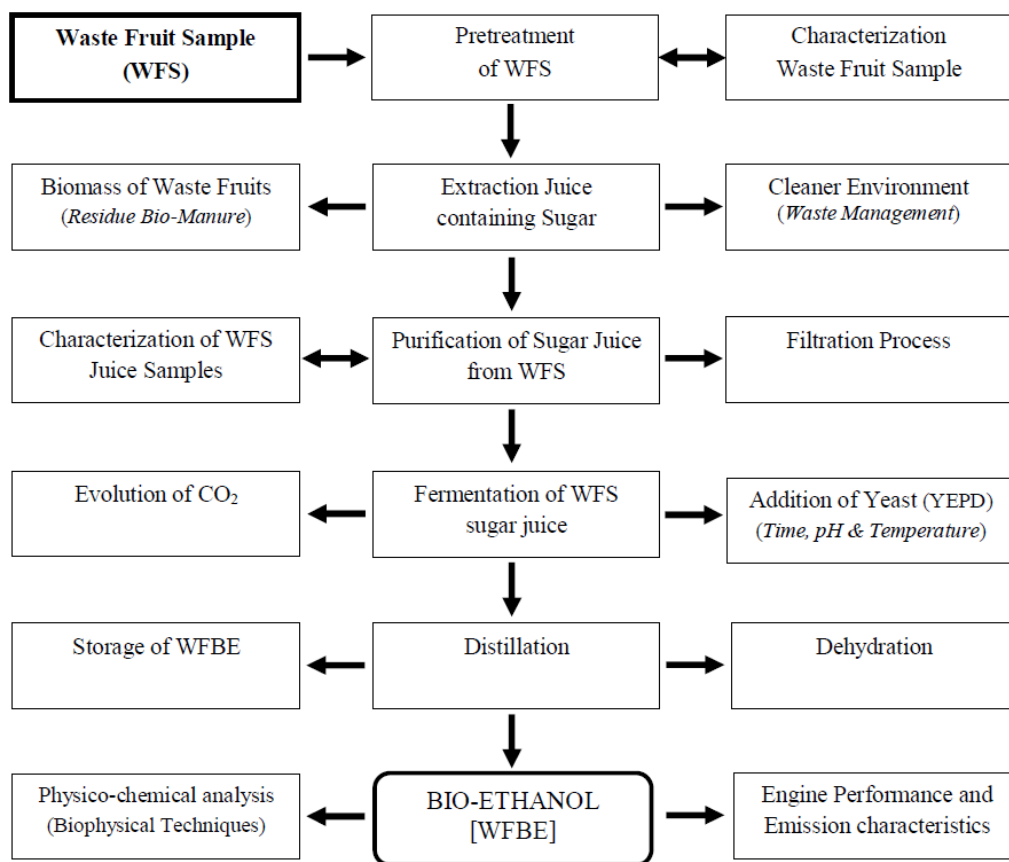


Fig 2. Experimental layout of Bio-ethanol production from waste fruit samples



Fig. 3. Waste Fruit Samples of individual category - A: Banana, B: Grapes, C: Mango, D & E: Sweet Lime, F: Papaya & Pineapple, G: Orange and H: Watermelon.

The alcohol (ethanol) fermentation is a process of conversion (transformation) of simple sugars such as glucose, fructose and sucrose to ethanol and carbon dioxide. Here, the fruit waste samples undergo fermentation in the presence of yeast and the sugars present in the fruits samples are converted to alcohol *i.e.*, ethyl alcohol and carbon dioxide. The chemical reaction involved in ethanol production is shown in Fig. 1.

However, the critical literature review on potential feedstock like, fruit wastes with its unit operation relating to its collection, processing, pretreatment, chemical characterization, yeast oriented fermentation including enzymatic hydrolysis will provide a gross picture about the need of scientific interference for bridging the gap on sustainable utilization of fruit wastes for bio-ethanol production. In addition, the earlier reports are also stimulating to optimize the bioethanol production by standardizing the procedure for biomass processing, experimentation and its post fact utilization behind the end-processes. In due course, the parametric analysis on interaction of the Engine test rig with fruit waste resulting in bioethanol was not clearly attempted. Hence, the reports on engine-biofuel interaction and their outcomes were also thoroughly cracked through explicit research literature works. Therefore, the reports of critical reviews on recent investigations were taken into consideration for the implementations of the proposed objectives (Table 1).

2. Recent investigations made on the production of Bio-ethanol from waste fruit samples

Furthermore, the comprehensive review and inferences of the previous research literature, gives a clear path for undertaking the current studies on potentials of fruit wastes resources which are found to be very crucial and most essential in order to facilitate the gap linking with sustainable ethanol production. Besides, the sustainable utilization of most promising and exceptional sources called fruit wastes to achieve bio-ethanol production with techno-economic approach in spite of environmental waste management. However, the research objectives are focused to design and improve a process and protocol in the experimentation, which would produce a sustainable transportation fuel using low/no cost, feed stocks (Fig. 2).

Finally, the amount of reviews on previous literature covering ethanol production from other types of feedstock like sucrose-based or starchy materials is more abridged. However, little effort has been made on ethanol production from pretreated enzyme saccharified fruit wastes by simple fermentation techniques.

Hence, the present study was framed to characterize the bio-ethanol derived from identified fruit waste samples of different class and parametric analysis relating to engine performance with emission characteristics at variable blending fractions of bio-ethanol with petrol-fuel.

3. MATERIALS AND EXPERIMENTAL METHODOLOGY

3.1. Raw materials and Microorganism

The waste fruit materials (Fig. 3.) such as, Banana, Grape, Mango, Sweet Lime, Papaya, Pineapple, Orange, Watermelon and Mixed Fruit samples were collected from a local market (Bengaluru and Ramanagara) places in large volume. The fresh waste fruit materials were brought to the laboratory and subjected for washing with demineralized water for removing any dirt particles physically adsorbed to the fruit materials. The washing was continued with acid/alkali for killing microorganisms from the surface area. Then, the washed waste fruit materials are air dried and maintain a constant weight of the samples in a hot air oven for a period of 8-12 hours. Further, the fruits were subjected for juice extraction using a suitable mechanical grinder. The Yeast sample was procured from authorized baking sectors and was subjected for re-hydration process with the addition of 20% distilled water followed by heating at 40°C in a water bath for about 20 minutes. The microbial growth media YEPD is used under aerobic conditions at temperature 300°C (Vishwakarma et al, 2014).^[13] The equipment used in the study are, hot plates, hot air oven, mechanical juice extractor, incubators, distillation unit coupled with hydrometer. The chemicals used in the studies were analytical grade obtained from authorized suppliers, Bengaluru.

3.2. Preparation of the Sample for fermentation process

The juice components of all the categorized waste fruits (Banana, Grape, Mango, Sweet Lime, Papaya, Pineapple, Orange, Watermelon



Fig. 4. Mixed Waste Fruit Samples

including Mixed Fruit samples were subjected for filtration using Whatman filter paper-1. Meanwhile, the fermentation system was set up and about 100 g of each sample was filled into 500 mL Schott bottle. Total soluble solid (TSS) value of each sample was taken before fermentation using Refractometer which is described in the analytical procedure (Khandaker *et al.* 2011 & 2012).^[14,15] The total sugar content in the waste fruit sample was analyzed using the standard procedure. The pH value of the samples was adjusted initially at room temperature using Hydrochloric acid prior to inoculation. Later, the pH was standardized to all samples tested.

3.3. Evaluation of Total sugars in the waste fruit samples

The juice component of all the waste fruit samples (Banana, Grape, Mango, Sweet Lime, Papaya, Pineapple, Orange, Watermelon and Mixed Fruit (Fig. 4)) were made into slurry using distilled water with a solid to liquid ratio of 10% (w/w). The Waste Fruit Sample (WFS) were subjected for stirring at room temperature at 200 rpm for 90 min. Then, the WFS were centrifuged at 3000 rpm for about 20 min. The supernatant fluid was then taken out and filtered using Whatman filter paper No.1 and the filtrate was subjected for quantitative evaluation of total sugar content in the samples by following the standard procedure (Dubois *et al.*, 1956).^[16] The amount of sugar was determined based on the colored aromatic complex formed between phenol and the carbohydrate at an absorbance of 490 nm. The quantity of sugar in the samples was analyzed and compared with a calibration curve using a standard reference of D-glucose at UV-Vis Spectrophotometer Cintra-5.

3.4. Preparation of the Inoculum and fermentation process

The yeast material was procured from CFTRI, Mysuru and the yeast inoculum was prepared in YEPD (Yeast Extract-Peptide-Dextrose)

Table 2. Bio-ethanol (%) obtained from different waste fruit samples with respect to Specific gravity

S.No	Waste Fruit samples	Specific Gravity	Ethanol Recovery (%)
1.	Banana	0.945	6.75
2.	Grape	0.846	11.85
3.	Mango	1.104	9.75
4.	Sweet Lime	0.872	13.86
5.	Papaya	1.032	8.45
6.	Pineapple	1.030	9.75
7.	Orange	1.050	7.85
8.	Watermelon	0.879	11.10
9.	Mixed Fruit	0.988	13.05

broth. The substrate of the fermentation system was subjected for inoculation with 1 mL culture broth / 100 mL substrate. The fermentation system of each sample including mixed waste fruit was allowed actual reaction for the period of 1 week without any interruption. The fermentation was carried-out at variable pH and temperature and reduced sugar content and yeast concentrations under agitation and immobilized conditions. The specific gravity of the waste fruit samples (WFS) were analyzed although the fermentation process using a hydrometer (Table 2). The fermentation process was ended by indicating the steady state of specific gravity of the WFS as the incubation period of fermentation varies for variable fruit juice samples (Behera *et al.*, 2010).^[17] The experiments for all parameters tested were done in triplicates.

3.5. Ethanol recovery by Distillation process

The fermentation process was performed as per the above procedure using yeast extract-peptone-dextrose. Later, the fermented broths were removed at 48 hours of interval and the contents were subjected for evaluation total sugar and ethanol. By employing the simple distillation process at a temperature between

Table 3. Bio-ethanol obtained from different waste fruit samples

S.No	Waste Fruit samples (100 mL)	Ethanol Concentration (mg/ml)	Ethanol Concentration ($\mu\text{g/ml}$)
1.	Banana	0.66	660
2.	Grape	0.53	530
3.	Mango	0.28	280
4.	Sweet Lime	0.36	360
5.	Papaya	0.49	490
6.	Pineapple	0.43	430
7.	Orange	0.35	350
8.	Watermelon	0.42	420
9.	Mixed Fruit	0.47	470

Table 4. Reducing Sugar content (g/L) in waste fruit samples with respect to time duration of fermentation process

S.No	Waste Fruit samples	Fermentation Time (Hrs.)		
		24 hrs	48 hrs	72 hrs
1.	Banana	53.85	50.93	48.68
2.	Grape	76.25	89.56	82.35
3.	Mango	51.39	53.55	50.42
4.	Sweet Lime	56.66	61.25	58.55
5.	Papaya	86.50	91.26	88.66
6.	Pineapple	57.65	52.48	51.44
7.	Orange	55.60	58.90	57.54
8.	Watermelon	114.55	168.60	141.44
9.	Mixed Fruit	63.55	61.20	60.55

75-96°C thereby, the mixture comprising of both ethanol and hot water was separated. The ethanol of 80% purity was obtained, on subjecting the same using rectifier units, finally 99.6% pure ethanol was achieved in Table 3 (Mandal and Kathale, 2012).^[18]

3.6. Estimation of reducing sugar content and Ethanol yield

Bio-ethanol yield was determined by the measurement of ethanol absorbance at 575 nm wave length, after conducting ethanol assay following the Dichromate Colorimetric Method (William and Darwin, 1950)^[19] using spectrophotometer. The absorbance values were compared to the ethanol standard graph and the percentage of ethanol was calculated. Glucose content was determined by DNS method (Miller, 1959)^[20] and the absorbance taken from each samples was compared to the standard curve of reducing sugar to calculate the sugar content (Table 4). Subsequently, the content of reducing sugars was determined by 3, 5-dinitrosalicylic acid. A standard curve was drawn by measuring the absorbance of known concentrations glucose solutions at 450 nm. The DNS reagent was consisted of 1% dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulphite and 1% sodium hydroxide. To measure glucose content, 3 mL of unknown glucose solution was filled into a test tube, followed by addition of 3 ml of DNS reagent. The test tubes were then heated in boiling water bath for 15 minutes. Exactly 1 mL of 40% potassium sodium tartrate solution was then added prior to cooling. All test tubes were cooled and then its absorbance was measured at 450 nm wave length.

3.7. Evaluation on Elemental composition of bio-ethanol derived from Mixed Fruit waste

The elemental composition of bio-ethanol derived from mixed fruit sample was evaluated by means of multi element oil analyzer (MOA II).

Table 5. Specifications of the Engine system

Make	Kirloskar model AV1
No. of Strokes per cycle	4
No. of Cylinders	single
Combustion chamber position	vertical
Cooling method	Water cooled
Starting condition	Cold start
Ignition technique	Compression ignition
Bore (D)	80 mm
Stroke (L)	110 mm
Rated speed	1500 rpm
Rated power	5 hp (3.72 kW)
Compression ratio	16.5:1

3.8. Viscosity and acid value analysis

Acid value was measured and for viscosity test, the samples were put in the beaker and heated up at 40°C and then measured by using viscometer. The viscometer was set at 30 rpm and then, spindle with the size of 66 was used (Hossain et al, 2010).^[2]

3.9. Gas Chromatographic (GC) analysis of bio-ethanol

The ethanol achieved in the fermentation broth was estimated by gas chromatography method. A computer related Nucon series gas chromatograph equipped with flame ionization detector (FID861) was employed for the separation and quantification of ethanol. A stainless steel column (5 m x 2 mm) was fitted into the instrument to provide on column injection. The column packing was Porapak Q. The detector and injector temperature was maintained at 200°C. The gas chromatograph was connected to an integrator and computer system to determine area of ethanol and internal standard peak (Gomez et al, 2014).^[21]

3.10. Performance Testing of Bio-ethanol using Engine Experimental Test Rig

In the study, an experimental engine test rig was developed by keeping the context of a direct injection diesel engine. The specifications of the diesel engine are given in Table 5. The engine was operated with cooling water and lubricating oil temperatures of 85-90°C. The engine was first operated on petroleum diesel with no load for few minutes at rated speed of 1500 rpm until it comes to the steady state conditions. Then the ethanol was used to obtain the baseline parameters at the rated speed by varying 0 to 100% of load on the engine with an increment of 20%. Later, the blends of ethanol obtained from waste fruit samples (WFE) blends were prepared by making different ratios. Further, the brake power is measured by a rope brake dynamometer. The exhaust emissions such as carbon monoxide, CO₂, NO_x, hydrocarbons and unused O₂ were measured by AVL Di-Gas 444 exhaust analyzer and the smoke opacity by AVL smoke meter 437°C for pure ethanol and all its blends with petrol separately under all load conditions. Further, the ethanol (99.5% pure) used to in different blending ratios in order to evaluate the performance parameters followed by emission properties respectively. The experimental set up consists of a diesel engine, engine test bed, and fuel and air consumption metering equipment, gas analyzer and smoke meter. The schematic diagram of the engine test rig is shown in Fig. 5.

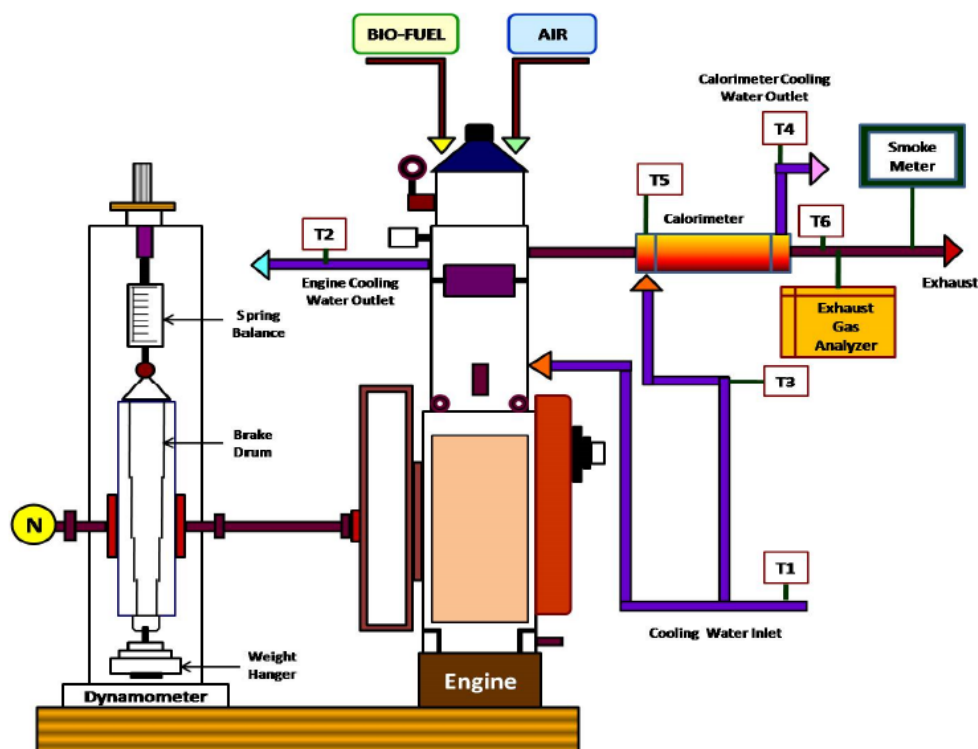


Fig. 5. Schematic diagram of Experimental Engine Test Rig for Bioethanol interaction

Table 6. Proximate composition (%) of waste fruit samples of individual category

S.No	Waste Fruit samples	Moisture	Dry Matter	Lipid	Crude Protein	Starch	ash
1.	Banana	78.56	28.25	1.42	5.82	0.68	3.78
2.	Grape	68.42	10.32	1.40	1.72	0.00	2.06
3.	Mango	83.36	18.45	1.51	8.31	0.54	6.45
4.	Sweet Lime	80.60	13.82	1.58	5.39	0.41	3.39
5.	Papaya	88.85	14.40	2.42	2.20	0.46	4.62
6.	Pineapple	55.45	8.33	0.60	6.23	0.00	1.85
7.	Orange	82.22	12.60	1.25	0.90	0.00	2.86
8.	Watermelon	91.66	11.96	0.65	2.40	0.00	1.60
9.	Mixed Fruit	96.77	36.76	1.39	4.25	0.28	3.61

Values are expressed on dry weight basis. All data are the mean of three replicates. Mean value followed by different letters in the same column differs significantly.

3.11. Statistical Analysis

The experimental design was completely randomized, with three replicates. All data were expressed as mean values \pm SE. The comparison between the mean values were tested using Duncan's new multiple range test and the ANOVA was also performed to find out the LSD ($p < 0.05$) using Number Crunch Statistical Software (NCSS, 2000).

4. Results and Discussion

In the present investigation, production of bio-ethanol from waste fruits samples of different categories such as, Banana, Grape, Mango, Sweet Lime, Papaya, Pineapple, Orange, Watermelon followed by mixed waste fruits were carried-out using yeast fermentation technologies. The waste fruit samples being generated at the local market regions are playing an important role in providing alternative cheap sources for ethanol production and the data of experimental observations are enunciated hereunder.

4.1. Characterization of waste fruit Biomass

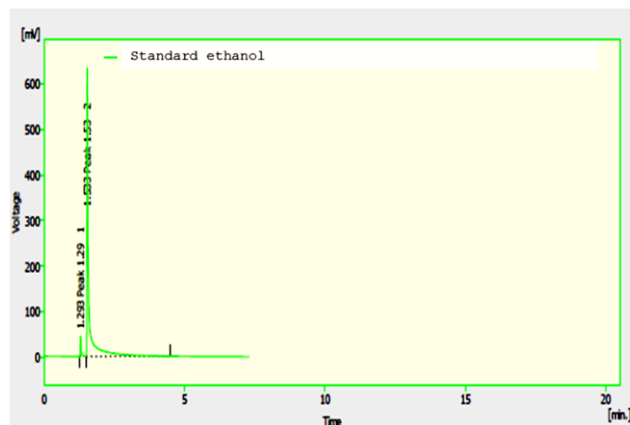
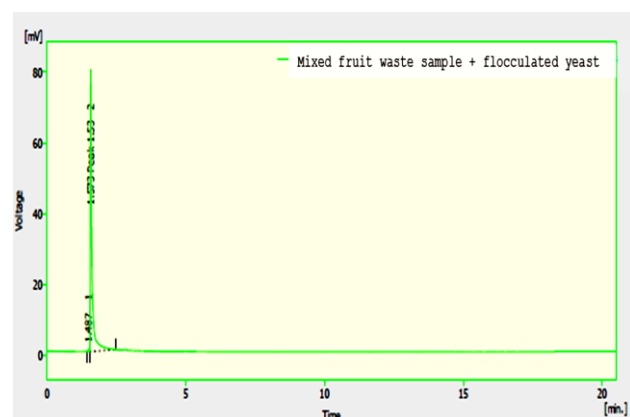
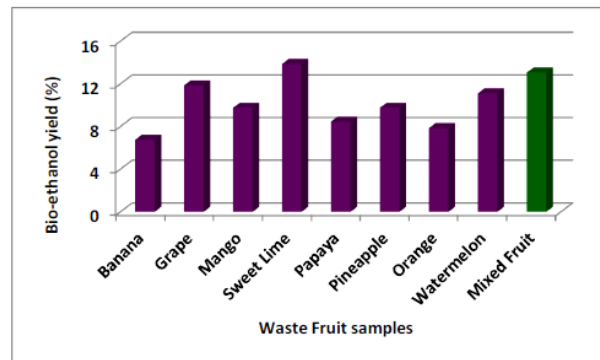
The most potential sources for sustainable ethanol production are Banana, Grape, Mango, Sweet Lime, Papaya, Pineapple, Orange and Watermelon reveals that, the maximum production of ethanol was noticed in mixed waste fruit samples using yeast fermentation which might be presence of diversified elemental compositions in the mixed waste fruit samples.

4.2. Proximate analysis

In the proximate analysis, watermelon had the highest moisture content 91.66% followed by papaya (88.85%), mango (83.36%), orange (82.22%) and the lowest moisture content of 55.45% was noticed in Pineapple waste fruit sample (Table 6). The high moisture contents of the identified waste fruits comparatively needs drying and facilitating better control of process variables with respect to enzymatic hydrolysis of the samples are concerned. Apart from this, the mixed waste fruit sample showed highest moisture content (96.77%) which is due to presence of diversified fruit wastes in a

Table 7. Effects of increasing Sugar concentration on Yeast growth in YEPD medium

S.No	Time (hrs.)	Sugar Concentration (%)					
		5	10	15	20	25	30
1	0	0.087	0.172	0.240	0.415	0.456	0.820
2	12	0.760	0.766	0.896	0.966	0.743	0.662
3	24	1.265	1.195	1.122	1.565	1.271	1.103
4	36	2.320	1.886	1.975	2.222	1.709	1.590
5	48	2.676	2.633	2.720	2.911	1.851	1.697
6	72	2.944	2.888	2.855	3.102	1.944	1.788

**Fig. 6.** Graph showing GC of Standard Ethanol**Fig. 7.** Graph showing Chromatogram of Bio-ethanol by mixed fruit samples using yeast strain *S.cerevisiae-198BIRD***Fig. 8.** Bio-ethanol yield (%) in different types of Fruit wastes samples

waste fruit samples. The results showed that the absorption bands slightly overlap due to the presence of different sugars in the substrate Figs. 6 & 7. The result may be attributed to the polysaccharides content present in each waste fruit sample formed a heterogeneous mixture of cellulose, hemicelluloses and pectin which are the main components of plant cell wall. Hence, the total sugar content in waste fruit samples is an imperative parameter facilitating the significant bio-ethanol production (Masuko et al, 2005).^[26]

4.4. Fermentation and Bio-ethanol production

The fermentation process was carried out using yeast which is known to play a very significant role with respect to various factors such as substrate concentration, temperature, pH and size of inoculums respectively. Therefore yeast cells are very crucial to optimize the fermentation conditions for achieving better quantities of ethanol with efficiency. However, *Saccharomyces cerevisiae* was used in this study that successfully converted cellulosic content in fruit samples to ethanol. There was a steady increase in pH of biomass during fermentation involving enzymatic hydrolysis of fruit wastes.

4.5. Role of Yeast and effect of sugar concentration on Ethanol recovery

The growth of *S. cerevisiae* was gradually increasing the concentrations of sugar showed an increase in optical density upto 20% sugar concentration in YEPD medium as shown in Table 7. However on increasing the sugar concentration beyond 20%, the growth was inhibited as shown by the optical density measured. Samples were taken every 12 hours for the study of growth kinetics. The growth was measured at 600 nm. The similar approach was also reported by Moaris et al (1996)^[27] where, the viability of *Saccharomyces* sp. in 50% glucose was observed and reported a viability of 10-98.8% in different strains of yeast.

common mass that may have contributed moisture in a considerable approach. The low cost source of dietary fiber composed mainly of hemicelluloses followed by pectin polysaccharides.

Similarly, the ash contents of the waste fruit samples showed that mango had higher ash content of 6.45% while, lowest ash content (1.60%) was recorded in watermelon. The content of lipids was high in papaya (2.42%) and the lowest lipid content (0.60%) was noticed in Pineapple. The significant presence of protein content in the waste fruit samples clearly; indicating that there is a low level of lignin in the samples that makes the substrate feasible during the hydrolysis process. This is in accordance with the earlier reports made by Ajila et al, (2007),^[22] Aguiar et al, (2008),^[23] Brooks, (2008)^[24] and Li et al, (2010).^[25]

4.3. Estimation of Total sugar content in Waste fruit samples

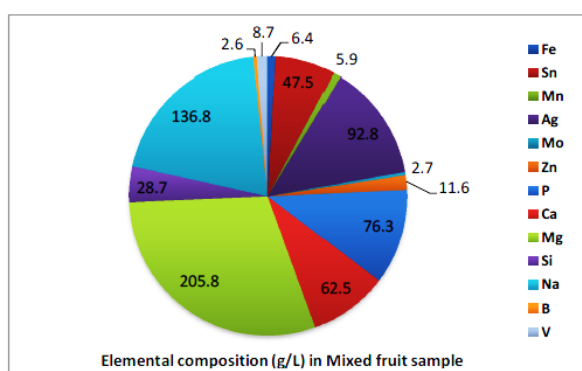
The developed complex absorbs UV-Vis light at 490 nm and the absorbance is proportional to the sugar concentration present in the

Table 8. Effect of pH on growth of Yeast and yield of ethanol from waste mixed fruit sample

S.No	Time (hrs.)	Growth of Yeast (OD)				Bio-ethanol yield (%)			
		pH5	pH6	pH7	pH8	pH5	pH6	pH7	pH8
1	0	0.265	0.265	0.296	0.252	0	0	0	0
2	12	0.455	0.925	0.925	0.825	0.850	2.42	3.64	3.52
3	24	1.105	1.765	1.556	1.756	3.66	7.96	4.45	6.82
4	36	1.386	2.255	2.050	2.250	6.80	9.65	8.45	9.43
5	48	1.596	2.662	2.188	2.610	9.45	12.88	10.35	10.76
6	72	1.658	2.770	2.250	2.766	10.75	13.60	11.02	11.22

Table 9. Effect of Temperature on growth of Yeast and yield of ethanol from waste mixed fruit sample

S.No	Time (hrs.)	Growth of Yeast (OD)				Bio-ethanol yield (%)			
		25°C	30°C	35°C	40°C	25°C	30°C	35°C	40°C
1	0	0.265	0.275	0.255	0.260	0	0	0	0
2	12	0.565	0.755	0.626	0.556	1.65	5.75	4.05	1.76
3	24	0.978	1.660	1.445	0.855	3.55	8.10	6.54	3.15
4	36	1.445	2.255	1.600	1.107	6.55	10.45	8.98	3.76
5	48	2.264	2.706	2.198	8.65	16.20	16.20	9.85	5.35
6	72	2.450	2.860	1.985	1.766	9.50	17.88	10.89	5.66

**Fig. 9.** Elemental compositions of Mixed Fruit waste sample.

The highest amount of bio-ethanol (11.35%) obtained at Grape Banana followed by significant amount of bio-ethanol Watermelon (11.10%); Pineapple (9.75%) and the lowest amount of bio-ethanol (5.75%) was recorded at banana whereas, the mixed fruit sample exhibited momentous bio-ethanol (9.05%) as compared to individual waste fruit category (Table 7 and Fig. 8).

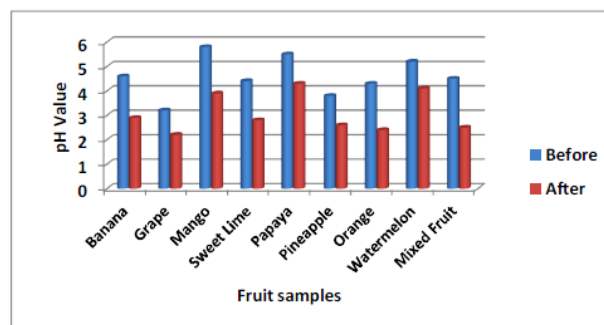
Fruit wastes are essentially destined for rich resources of fermentable sugars which can be a potential approach for ethanol production (Itelima et al., 2013).^[28] The previous reports are also in concurrence with the results where the fruit samples can replace the gasoline as sustainable transportation fuel (Jimoh et al., 2009).^[29] The overall expression of bio-ethanol recovery was found to be most significant in which the fermentation reaction is effectively monitored by the strain of yeast in *S. cerevisiae* in all the waste fruit samples including mixed waste fruits samples (Shilpa et al., 2013).^[9]

4.6. Elemental Compositions in Mixed fruit sample

In the analysis, it can be seen that there are several additive metals present in waste fruit samples at concentration of yeast (1 g/L) which is represented in the Fig. 9. The additive metal consists of Zinc (Zn), Phosphorus (P), Calcium (Ca), Magnesium (Mg) and Boron (B) is present in the sample with respect to yeast concentration. The highest elemental concentration of magnesium (205.8 g/L) and Sodium (136.8 g/L) Phosphorus (76.3 g/L) and the lowest concentration of Boron (2.6 g/L) were observed (Fig. 9). The results

Table 10. Physico-chemical characteristics of Absolute ethanol, Waste fruit ethanol and gasoline

S.No	Properties	Produced bioethanol	Produced bioethanol	Gasoline
1	Chemical Formula	C ₂ H ₅ OH	C ₂ H ₅ OH	C ₈ H ₁₇
2	Molecular Weight	46	46	113.2
3	Specific gravity	0.79	0.83	0.74
4	Absolute Viscosity (cp)	1.2	1.31	0.56
5	Flash point (K)	290.15	302.15	228
6	Fire point (K)	299.15	307.15	234
7.	Lower Heating Value (MJ/kg)	27	23.4	43.5

**Fig. 10.** pH value in different Fruit wastes samples at before and after fermentation process.

of this study showed that there were no dangerous elements in bio-ethanol from pineapple and orange wastes based on American Society of Testing and Materials (ASTM) and ASTM D5806 (Williams et al., 2009).^[30] Mineral concentration in fruit and vegetable juice varies with growing condition, growing medium, cultivar and harvesting maturity. Moneruzzaman et al. (2008)^[31] reported that fruit physiological characteristics and quality of juice depend on the stages of fruit maturity.

4.7. Optimization parameters for Ethanol production

4.7.1. Effect of pH on ethanol yield

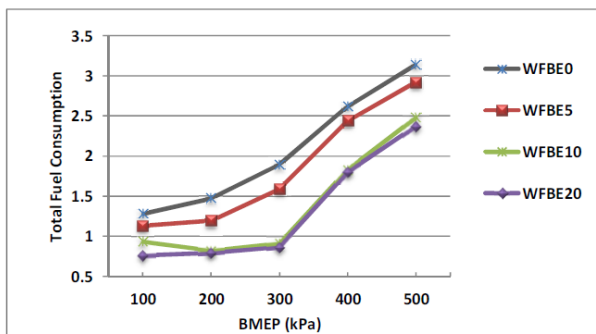
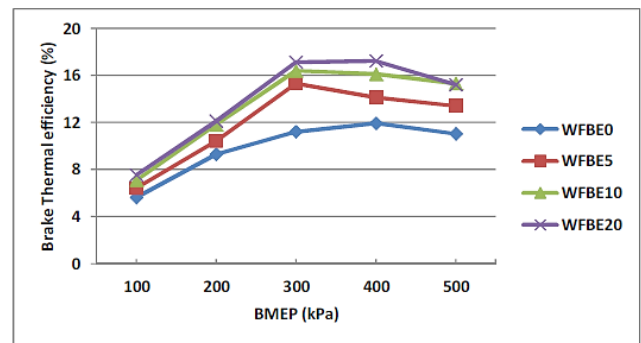
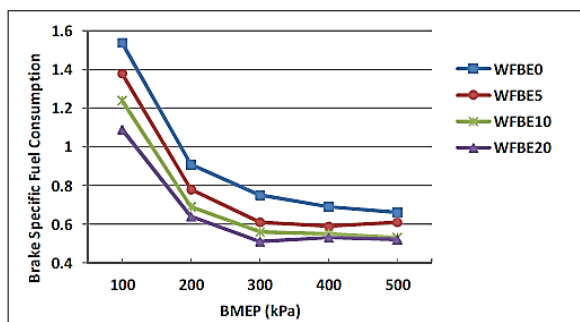
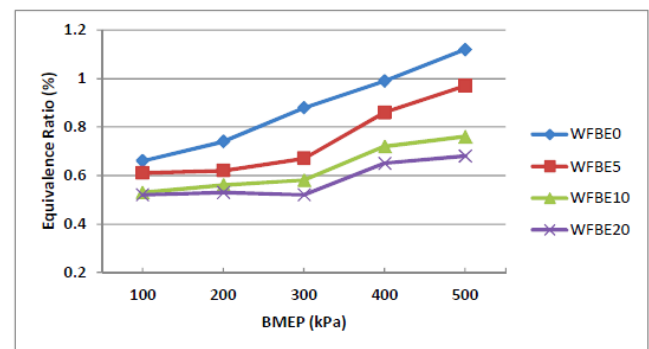
In the analysis, the initial sugar concentration of 20% and optimum temperature of 30°C was selected for further studies and subjected to pH treatments 5, 6, 7 and 8 respectively. The

Table 11. Bio-ethanol production from mixed fruit samples

S.No	Production of Bio-ethanol from mixed fruit samples under optimum conditions with <i>S.cerevisiae</i> -198BIRD				Bio-ethanol Productivity $\text{g.l}^{-1}.\text{h}^{-1}$	Bio-ethanol Production (%)
	24 h	48 h	72 h	96 h		
1	78.189	149.137	129.579	120.673	3.156	80.3204
2	78.189	149.137	129.579	120.673	3.156	80.3204

Table 12. GC-MS analysis of Standard Ethanol

No.	Retention Time (min)	Area (mV.s)	Area (%)	Amount (μl)	Amount (%)
1.	1.293	95.936	3.4	N/A	N/A
2.	1.533	2677.936	96.6	99.000	100.0
Total		2773.494	100.0	99.000	100.0

**Fig. 11.** Total Fuel Consumption (TFC) Vs BMEP @ 2000 RPM**Fig. 13.** Brake Thermal Efficiency (BTE) Vs BMEP @ 2000 RPM**Fig. 12.** Specific Fuel Consumption (BSFC) Vs BMEP @ 2000 RPM**Fig. 14.** Volumetric Efficiency (VE) Vs BMEP @ 2000 RPM

fermentation yielded low ethanol content at pH 5. The significant results were obtained at pH 6 where superior ethanol production was noticed (Table 8 and Fig. 10). The increase in ethanol with an increase in pH range from 4.0-5.0 was observed in the earlier reports and the optimum yield was achieved in the pH range between 5.0 to 5.5 (Yadav *et al.*, 1997).^[32] Hence, pH 6 was preferred based on the fermentation efficiency for further experimentation.

4.7.2. Effect of temperature on Ethanol yield

The temperature is a crucial factor which directs the reaction mechanism in the bio-ethanol production, and is one of the major constraints that determine the ethanol production. The optimum temperature for ethanol fermentation was assessed by keeping the solutions at variable temperatures of 25, 30, 35 and 40°C with 20% initial sugar concentration. The parameters relating to growth of *S. cerevisiae* and the ethanol yield were concurrently focused. The samples were withdrawn at every 12 hours and the fermentation was carried out for the duration of 48 hours. A low ethanol yield of 6.8% was observed at 25°C in 48 hours. The bio-ethanol yield was

found to be highest at 30°C and turned out to be 11% (Table 9). However, the growth as well as concentration of alcohol decreased with increase in the temperature beyond 30°C. This decrease was pronounced at 40°C so 30°C was selected as optimum temperature for ethanol production (Morimura *et al.*, 1997).^[33] The temperature tolerance was also noticed as the fermentation of waste fruit sugar was possible at 35°C when sugar concentration was 20% (w/v) with no fermentation when sugar concentration was 22% (w/v).

4.7.3. Physico-chemical properties of bio-ethanol

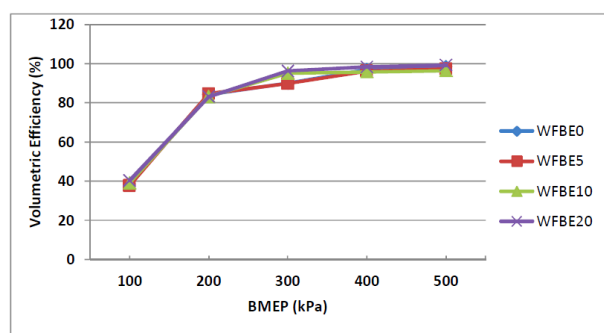
The bio-ethanol obtained from fruit peels showed optimum characteristics for use as automobile fuel. The physical properties of bio-ethanol were compared with that of absolute alcohol (Commercial ethanol) and gasoline in Table 10. The variation could be attributed to moisture content retention in the produced bio-ethanol after dehydration. The produced bio-ethanol has been represented for the samples of individual categories of waste fruits followed by mixed waste fruit samples respectively exhibited remarkable physico-chemical properties which is slightly outstanding

Table 13. GC-MS analysis of mixed fruit waste ethanol sample

No.	Retention Time (min)	Area (mV.s)	Area (%)	Amount (μ l)	Amount (%)
1.	1.487	1.119	0.4	0.000	0.0
2.	1.573	317.386	99.6	11.733	100.0
Total		318.505	100.0	11.733	100.0

Table 14. Emission analysis in response to Fuel Engine-ethanol interaction

Emission (ppm) and Fuel consumption (ml/sec)	Standard or 100% gasoline: WFBE0	5% Ethanol & 95% gasoline: WFBE5	10% Ethanol & 90% gasoline: WFBE10	20% Ethanol & 80% gasoline: WFBE20
CO ₂	9	8.1	8.1	8.2
CO	6.2	6.0	6.0	6.0
SO _x	902	335	280	294
NO _x	66	27	22	24
HC	75	35	27	29
Fuel consumption	1.8	1.4	1.2	1.5

**Fig. 15.** Equivalence Ratio (EqR) Vs BMEP @ 2000 RPM

as compared to earlier reports (Yücesu et al, 2006^[34] and Kang et. al, 2015).^[6] This has also been endorsed in the enhanced production of Bio-ethanol from mixed fruit samples under optimum conditions with respect to different time durations which is affected by *S.cerevisiae* 198BIRD (Table 11).

4.7.4. Gas Chromatographical (GC) analysis

The produced ethanol showed extreme purity using GC-MS technique in comparison with a standard. The commercial ethanol was used as a reference for GC analysis as shown in Table 12 & Fig. 6. The compound eluted from the column at a retention time of 1.533 min. The bio-ethanol obtained from fruit peels showed a retention time of 1.487, 1.573 minutes with a deviation in retention time (Table 13 & Fig. 7).

4.7.5. Performance Testing during Engine-fuel interactions

The interaction of engine with the blends of bio-ethanol prepared by mixing of ethanol with pure gasoline in the following proportions (5%, 10%, 20%) by volume: 5% WFBE (Waste Fruit Bio-Ethanol) and 95 % petrol, 10% WFBE and 90 % petrol and 20% WFBE and 80 % petrol respectively. The experiments were conducted over the same range of engine loads at rated speed. The brake power is measured by a rope brake dynamometer. The exhaust emissions such as carbon monoxide, CO₂, NO_x, hydrocarbons and unused O₂ are measured by AVL Di-Gas 444 exhaust analyzer and the smoke opacity by AVL smoke meter 437°C for pure ethanol and all its blends with petrol separately under all load conditions. The results of the engine operating on various WFBE blends are compared with the baseline

parameters obtained during engine fueling with pure petrol at rated speed of 2000 rpm (Fig. 5).

The variation in Total Fuel Consumption (TFC) with BMEP (Brake Mean Effective pressure) at an engine speed of 2000 rpm has been demonstrated where the fuel consumption was found to increase for each blend with an increase in BMEP (Fig. 11). At the maximum fuel consumption was noticed at WFBE0 at all points of BMEP and when the bio-ethanol content was increased in the blend of projected ratio, the total fuel consumption was reduced. The Brake Specific Fuel Consumption (BSFC) with BMEP at an engine speed of 2000 rpm was analyzed (Fig. 12), where the increased BMEP striving the BSFC in decrease mode at all the four blends. Later, at a given BMEP, the BSFC was found to be decreased as the ethanol blending ratio was increased. As a consequence, bio-ethanol showed a reducing effect on the BSFC.

Further, the BTE (brake thermal efficiency) was found to increase with an increase in BMEP as well as bio-ethanol blending content. The peak efficiency of 17-18% was noticed for the WFBE10 blend (Fig. 13). The thermal efficiency may possibly be enhanced by increasing the compression ratio which could be accommodated for bio-ethanol blends since ethanol has a higher octane number rather than gasoline (Yücesu, et al, 2006).^[34] At last, the increase in volumetric efficiency was noticed with increase in the BMEP for all the blends. In due course, the increased content of bio-ethanol improved the volumetric efficiency. Therefore, at the peak of BMEP, the effect of bio-ethanol content on the volumetric efficiency was found to be more prominent for the WFBE10 blend (Fig. 14).

The variation of the equivalence ratio with the BMEP at an engine speed of 2000 rpm was recorded where, the equivalence ratio of WFBE0 (100% gasoline only) which placed within lean limits at the lowest BMEP. The increased BMEP has a tendency of the mixture attaining at-Stoichiometric strength on the peak of BMEP (Fig. 15). The bio-ethanol blends WFE5, WFE10 and WFBE20 demonstrate an equivalence ratio less than unity i.e. the mixtures remained lean at all values of the BMEP. The blend of WFBE10 and WFBE20 showed a significant BMEP, hence posturing a defensive approach of flame put out even at cold start conditions. The similar expressions were also reflected in the previous reports (Celik, 2008^[35] and Prashanth et al, 2007).^[36]

Hence, for increasing the content of bio-ethanol beyond 20%, some modification in the air intake system was deemed necessary to

regulate the rate of air supply into the engine in correlation with the operational air-fuel ratios. The comparison of the stoichiometric air-fuel ratio with the actual air-fuel ratio has been represented along with the density and lower heating values of different blends (Tables 10, 14).

4.7.6. Emission analysis on Bio-ethanol-petro-fuel blending

The CO₂ (carbon dioxide) and CO (carbon monoxide) emissions were reported to be lower than the petro-fuel. The effect of using bioethanol derived from waste fruits decreased the NO_x (nitrogen oxides) emissions at medium engine speeds, i.e. approximately 30.0%. In addition, the lesser NO_x emission was also attributed to the reduction of cetane number of the diesel-bio-ethanol blended fuels' cetane number as the amount of bioethanol increases. However, the emissions of NO_x were found to increase gradually at low speed (1600 rpm), high load; high speed (2400 rpm), medium load conditions. It was found that the combustion performance and emissions of the diesel engine very much depend on the fuel, its emulsion combination types and engine operating conditions (Table 14).

5. Conclusions

In present investigations, the bio-ethanol was achieved from different waste fruit samples such as, Banana, Grape, Mango, Sweet Lime, Papaya, Pineapple, Orange and Watermelon followed by mixed waste fruit samples respectively, which were procured from local market areas of both Bengaluru and Ramanagara districts (Karnataka), India. The yeast strain (*Saccharomyces cerevisiae*) was obtained from 'Hamsa Research foundation', Tumakuru, India.

The fermentation process was executed as per the standard procedure and after 7-10 days, it was feasible to achieve considerable bio-ethanol by individual waste fruit category such as waste Grape fruits (11.35%) followed by Watermelon (11.10%), Pineapple (9.75%) and mango (8.65%) respectively, whereas, the mixed fruit waste recorded significant amount of bio-ethanol (10.95%).

After accomplishing successful fermentation processes the other dimension of bio-ethanol production, revealed that, 730 µg/ml; 640 µg/ml; 510 µg/ml; 470 µg/ml; 460 µg/ml and 420 µg/ml of ethanol from 100 mL of fruit juices of Grape, Banana, Orange, Papaya and Sweet Lime and watermelon respectively after distillation and maintaining a pH of 5.5 and temperature of 30°C. Apart from these, the mixed waste fruit samples was exhibited superior bio-ethanol (590 µg/ml) as compared to individual category of waste fruit samples where the physio-morphological analysis is very complex compared mixed fruit approach. The produced ethanol showed extreme purity using GC-MS technique in comparison with a standard.

In the engine interactions, the bio-ethanol blends WF5, WF10 and WF20 demonstrates that, the TFC with BMEP followed by BSFC with BMEP, BTE with BMEP at an engine speed of 2000 rpm may possibly significant in their expressions and found to be superior by increasing the compression ratio which could be accommodated for bio-ethanol blends since ethanol has a higher octane number

rather than gasoline. In addition, at the peak of BMEP, the effect of bio-ethanol content on the volumetric efficiency was found to be more prominent for the WFBE10 blend. Further, the equivalence ratio of WFBE0 (100% gasoline only) was placed within lean limits at the lowest BMEP. The increased BMEP has a tendency of the mixture attaining at-stoichiometric strength on the peak of BMEP.

The following are conclusions drawn on the performance of interaction between engine-bio-ethanol blends with gasoline with the diesel engine system.

- The brake thermal efficiency increased with an increase in ethanol content as well as an increase in BMEP's.
- TFC increased while BSFC reduced with the increasing ethanol content at different BMEP's.
- The volumetric efficiency was found to rise with the ethanol content at different BMEP's.
- There was a drastic reduction in the equivalence ratio especially at lower BMEP's which set a limitation on the maximum content of ethanol that could be used for blending with gasoline.

Further, the engine-fuel interaction showed emission parameters like, hydrocarbon, NO, CO and CO₂ content were significantly lower in WFBE5 (5% - with 95% gasoline), WFBE10 (10% bioethanol with 90% gasoline) and WFBE20 (20% bio-ethanol with 80% gasoline) than in WFBE0 (100% gasoline) having less fuel consumption.

However, the Waste fruit samples (WFS) and mixed waste fruit samples (MWF) sources are found to be an alternate feed stocks for fossil fuels and producing more promising bio-ethanol with an eco-friendly approach with no release of toxic gases to the environment. Since the available sources are having no cost, only the action plan of proper collection of material, segregation, processing etc. are involved in this line of bio-ethanol production which has been put into effect as sustainable bio-ethanol production. In addition, the leftover residue generated after the processes as a value addition can be utilized as 'biomanure' which can be recommended for the nursery plantation on a profitable basis. On the other hand, optimization of substrate concentration and other environmental conditions are required for an industrial application. At the outset, the bio-ethanol from waste fruit sources can be a very good substitute for reducing drudgery and the dependency on conventional fossil fuel resources.

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Conflicts of Interest

The authors declare no conflict of interest.

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