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Potentiality of *Saccharomyces Boulardii* in Fermentation of Bio-Ethanol Derived from Fruit Wastes

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ABSTRACT

An astronomical increase in vehicular usage has led to the depletion of existing fossil fuels and forced researchers to find alternative sources of fuels. Extensive studies are utilizing *Saccharomyces cerevisiae* for bioethanol production. Therefore, in the present study, the potential application of *Saccharomyces boulardii* derived bioethanol from fruit wastes was explored. The orange, sweet lime, and banana peels were subjected to various pre-treatment procedures and used as substrates for yeast mediated fermentation. The cellulosic de-linearization of fruit peels induced by alkaline and steam pre-treatments was analysed using Fourier Transform Infrared Spectroscopic analysis. The chemical nature of fruit waste-derived bioethanol was compared with commercial ethanol using gas chromatographic analysis. Bioethanol used in ratios 0%, 4%, 8%, 12% by volume as part of fuel blends were subjected to performance testing. This testing process was carried out on a single-cylinder, four-stroke spark-ignition engine and the effect of bioethanol content (by volume) on the operational parameters like total fuel consumption, brake specific fuel consumption, brake thermal efficiency, volumetric efficiency, and equivalence ratios were assessed. FTIR spectra showed significant changes in the chemical structure of fruit peels due to pretreatments. The bioethanol yields increased up to 24 h of fermentation after which there was a steady decline. These results were confirmed by an increase in pH of the biomass after 24 h up to 72 h. Enzymatic hydrolysis of fruit peels resulted in higher bioethanol yield as compared to acid treatment. The fruit derived bioethanol showed ideal physicochemical characteristics for use as automobile fuel. The same showed decent performance when tested on a four-stroke, spark ignition engine.

Keywords:

Fruit waste; Bioethanol; Gas Chromatography; FTIR; Engine performance

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1. Introduction

Fuel is a major requirement of mankind for a wide range of applications ranging from transportation, electricity, domestic gas, nuclear to geothermal energy [1–3]. Fossil fuels, such as diesel, petroleum, gasoline, and kerosene have been utilized for centuries as the principal form of energy in automotive industries [4]. There is a steady depletion of liquid fuel reserves due to unlimited consumption and increasing demand in the transportation sector [5]. Fossil fuels are expensive, emit greenhouse gases (GHG), difficult to handle and are less efficient over a longer time [6]. Till date, bio-hydrogen, biodiesel, bioethanol, biogas, methanol, butanol, natural gas, liquefied petroleum gas (LPG) and fatty acid methyl ester (FAME) have found routine application as replacements for conventional fuels [7]. But research focus on the production of biofuels like bioethanol and biodiesel has been of prime significance in the current era of depleting fuels. Biofuels are mainly classified into four generations- first (feedstocks-animal fats, vegetable oils), second (lignocellulosic biomass), third (algae-based) and fourth (solar fuels, synthetic biology, metabolic engineering approaches) [8]. Ethanol derived from lignocellulosic biomass have a high octane number, generates low carbon emissions and can be blended with gasoline and diesel to improve their properties [9]. These also require fewer additives and release reduced aromatic derivatives in the process [10]. Lignocellulosic materials are predominately used as starting material for ethanol production considering their minimal energy requirement, easy availability, lower cost of transportation, and higher fuel yields [11]. There are different methods of bioethanol production- fermentation involving common lignocellulosic material containing agricultural wastes like sugarcane bagasse, fruit peels [12], in addition to molasses [13] and plant pod husks [14]. There are three basic steps for conversion of plant-based substrates to final fuel- i) pretreatment, ii) hydrolysis and iii) fermentation [15]. The process of pretreatment is necessary for loosening the tightly bound cellulosic matrix (composed of mainly cellulose, hemicellulose, and lignin) by breaking intercalating glycosidic bonds which in its native form is not susceptible to enzymatic hydrolysis. Therefore, these fibers are subjected to various forms of pre-treatment, i) Physical methods included microwave, ultrasonic vibrations, high-temperature pyrolysis, ii) chemical methods primarily focused on acidic, alkali treatment, iii) physicochemical methods involved steam, and iv) biological method used enzymatic hydrolysis [16]. Hydrolysis breaks down macromolecules into simple sugars such as hexose, mannose and are mainly of two types- acidic and enzymatic. This process reduces cellulose crystallinity and gives an increased surface area for catalytic reaction. Acidic reaction results in the conversion of cellulose into glucose and hemicellulose to xylose components [17]. Therefore, enzymatic hydrolysis is a better option for the conversion of lignocellulosic biomass as it is cost-effective, less-corrosive, and results in a higher fuel yield [18]. There are two strategies of enzymatic hydrolysis for fermentation- i) separate hydrolysis and fermentation (SHF) and ii) Simultaneous saccharification and fermentation (SSF). In SHF, commercially procured enzymes like cellulases and hemicellulases break down cellulose and hemicellulose in the pre-treated substrate at approximate temperatures of 50 °C. However, in SSF, the pretreated raw material is incubated along with the inoculated microorganism in the fermentation tank predominately at temperatures ranging from 28 to 37 °C [19]. Fruit derived wastes are inedible, lead to bad odor and its open disposal leads to environmental fallouts. These fruits are rich in complex sugars such as pectin, cellulose, hemicellulose [20] and therefore, they can be converted into bio-based fuels through enzymatic fermentation reactions. To date, individual fruit peels have been fermented by cellulase/hemicellulases produced by *Saccharomyces cerevisiae* [21] for bioethanol generation. Therefore, in the present study, fruit wastes were separately subjected to enzymatic hydrolysis by α -amylase followed by microbial fermentation using *Saccharomyces boulardii*.

2. Methodology

2.1 Production of Bioethanol

2.1.1 Pretreatment of raw materials

Fresh fruit peels of orange (*Citrus sinensis*), sweet lime (*Citrus limetta*) and banana (*Musa acuminata*), cultivated in agricultural farms of Udupi were procured locally from a fruit shop in Manipal. They were refrigerated for 6 h after which they were shredded to obtain fine-sized particles ($\sim 75 \mu\text{m}$) to increase the available surface area for the pre-treatment reactions. The pilot-scale study involved taking the shredded peels (100 g each of the three different fruits) in separate sterile beakers and subjecting them to steam and alkaline pretreatments. The steam conditioning involved exposure of fruit peels to 160°C (steam) at 15 psi for 30 min in an autoclave. The pretreatment removed value-added product limonene usually present in citrus fruit peels [22]. This limonene inhibits the fermentation process due to its antimicrobial activity [23]. For alkaline treatment, 100 mL of 2 M NaOH (small scale), 500 mL of 2 M NaOH (large scale) were added to the respective beakers containing fruit peels. These pretreatments loosen the cellulosic matrix of fruit peels making them susceptible to enzymatic hydrolysis. FTIR analysis was used for evaluating the structural changes in fruit peels due to steam and alkaline pretreatments. The fruit peels after pretreatments were dried in a hot air oven at 70°C for 48 h. The dried peels were ground into fine powder using a laboratory blender and stored at 4°C (Figure 1). 10 mg of individual fruit powder was used in potassium bromide discs for sample processing. The sample spectra were analyzed using Jasco FT/IR-6300 (United States) in the range from 400 to 4000 cm^{-1} at a resolution of 0.07 for 100 scans.



Fig. 1. Treated fruit peels (a) Orange (b) Sweet lime (c) Banana

2.1.2 Hydrolysis treatment

The acidic hydrolysis treatment involved the addition of 100 mL of distilled water to the pre-treated samples to maintain the hydration. 50 mL of dilute sulfuric acid (10 % concentration) at pH 2 was added followed by sterilization at 120°C , 15 psi for 30 min. At this stage, the pH of peels was in the acidic range between 1 to 2. Therefore, the pH of peels was adjusted to 7.0 with 2 M NaOH for neutralization. For enzymatic hydrolysis, the acetate buffer was prepared by adding 90 mL of acetic acid and 170 mL of sodium acetate to 240 mL of distilled water. 15 mg of the enzyme (α -amylase) (procured from Sisco research laboratories, Mumbai, India containing 10-25 units/mg solids) was dissolved in 3 mL of distilled water to prepare the final enzymatic solution. 100 mL of acetate buffer was added to 15 g of pretreated peels and $15 \mu\text{L}$ of the enzyme was added to this in three 500 mL breakers (banana, orange, and sweet lime) respectively. The sealed beakers were incubated for 24 h at 40°C under shaking at 175 rpm. After overnight incubation, the samples were boiled for 2 min in

a heating mantle and centrifuged at 5000 rpm for 10 min at 37 °C. The concentrated samples were then transferred to three-necked round bottom flasks and sterilized in an autoclave at 120 °C at 15 psi for 30 min.

2.1.3 Fermentation

Lyophilized yeast powder (commercially available as gNorm) containing *Saccharomyces boulardii* was procured locally. 125 g of yeast (*S. boulardii*), 3 g of dextrose and ammonium sulfate were added to the hydrolyzed samples as carbon and nitrogen sources respectively. 4000 mL of sterilized lukewarm water was added to it and the solution was incubated for 7 d. The pH of fermentation solution was monitored continuously to evaluate the extent of bioethanol production. The final bioethanol was separated from the crude biomass after fermentation by using a vacuum distillation setup with rotor evaporator (Equitron Evator Rotary Evaporator fitted with a Refrigerated Circulating Bath from Medica Instruments, India). The sample solution was poured into the clean round bottom flask connected to a condenser with the help of a Keck clamp. The cooling bath was turned on and set at 15 °C. The vacuum pump was powered, and the hot bath was filled with enough water to accommodate the round bottom flask. The hot bath, as well as the evaporator, were switched on and the desired rotation of condenser was set to 100 rpm. The timing of the operation was set manually in the evaporator display. The hot water bath was maintained at a maximum temperature of 60°C. After the operation, the round bottom flask along with the other components was allowed to cool down. The bioethanol obtained was then subjected to physiochemical analysis. The specific gravity of the bioethanol was determined. Redwood viscometer was used to determine the absolute viscosity. Cleveland Flash and Fire point tester was used to determine the flash and fire points of bioethanol. A bomb calorimeter was used to measure the lower heating values of the bioethanol.

2.1.4 Gas Chromatographic (GC) analysis of bioethanol

The chemical nature of obtained liquid was confirmed using a Chemito GC 8610 gas chromatograph (Chemito Instruments, India) fitted with flame ionization detector (FID 861) using a capillary column (OV-17) for separation and IRIS 32 software for analysis. The carrier nitrogen gas was utilized at a flow rate of 2 mL/min. The oven temperature was set at 250°C while the injector and detector temperatures were at 180 °C and 300 °C respectively.

2.2 Performance Testing of Synthesized Bioethanol on Spark Ignition Engine

The bioethanol was tested on a four-stroke, single-cylinder, spark-ignition engine setup coupled with brake drum dynamometer. The engine parameters have been shown in Table 1. Using pure ethanol as the sole fuel was deemed infeasible since the fuel intake system and the engine had to be subjected to certain modifications. Hence, bioethanol in low concentrations was blended with gasoline for which no modifications of the engine or the fuel system was necessary [24]. Bioethanol in 0%, 4%, 8%, 12% by volume (E0, E4, E8, and E12) was blended with gasoline to give the final test fuel. The numbers succeeding E represent the percentage amount of bioethanol by volume. The engine was started and allowed to reach its optimum operating temperature. The engine speed was attuned to 2000 rpm during the performance test. The load on the dynamometer was set to 2.25 kg, 4.5 kg, 6.75 kg and 9 kg respectively to correspond to engine load fraction of 25%, 50%, 75%, and 100%. An orifice-meter placed in the air intake line across which a U-tube manometer was connected for measuring the rate of the air intake. A burette fitted with a three-way cock was used to measure

the time taken for 20 mL of the fuel to get consumed from which the fuel consumption rate was obtained. Eq. (1)-(4) were used to compute the brake power, total fuel consumption, specific fuel consumption, brake thermal efficiencies respectively at the corresponding engine load fractions. Eq. (5)-(10) were used for finding out the volumetric efficiencies. The brake means effective pressures were determined using Eq. (11). Eq. (12)-(15) were used to determine the actual air-fuel ratios. The stoichiometric air-fuel ratios for the different blend types were determined from combustion equations shown in Appendix A. The equivalence ratio was found out from Eq. (16).

$$BP = \frac{2\pi \times 2800 \times 0.1 \times W}{60000} \quad (1)$$

$$TFC = \frac{3600 \times V \times \rho}{1000 \times t} \quad (2)$$

$$BSFC = \frac{TFC}{BP} \quad (3)$$

$$\eta_{b,th} = \frac{3600 \times BP}{TFC \times Q_{CV}} \times 100 \quad (4)$$

$$\rho_a = \frac{P}{RT} \quad (5)$$

$$h_a = \frac{h_w \rho_w}{\rho_a} \quad (6)$$

$$Q_r = 60 \times C_d \times A_0 \times \sqrt{2gh_a} \quad (7)$$

$$Q_s = \frac{Q_r T_s}{T_r} \quad (8)$$

$$V_s = \frac{LAN}{2} \quad (9)$$

$$\eta_{vol} = \frac{Q_s}{V_s} \quad (10)$$

$$BMEP = \frac{BP \times 60000}{V_s} \quad (11)$$

$$\dot{m}_s = Q_r \times \rho_a \quad (12)$$

$$\dot{m}_a = Q_r \times \rho_a \quad (13)$$

$$\dot{m}_f = \frac{TFC}{60} \quad (14)$$

$$\left(\frac{A}{F}\right)_{Ac} = \frac{\dot{m}_a}{\dot{m}_f} \quad (15)$$

$$\Phi = \frac{\left(\frac{A}{F}\right)_S}{\left(\frac{A}{F}\right)_{Ac}} \quad (16)$$

Table 1
 Engine Specifications

Parameter	Specification
Bore (mm)	70
Stroke (mm)	66.7
Brake Drum Radius (mm)	100
Compression Ratio	9:1
Fuel System	Carbureted
Rated Maximum Speed (rpm)	2800
Rate Brake Power (kW)	2.5

3. Results

3.1 Characterization of Lignocellulosic Biomass

FTIR spectra showed variable compositional changes in the cellulosic content of fruit peels before and after steam, alkali pretreatments (Figure 2(a), Figure 2(b) and Figure 2(c)). The standard spectrum for untreated cellulose, hemicellulose, and lignin are well established in the latest research [25]. The untreated banana peels resulted in peaks approximately closer to 1233 cm^{-1} -C-O stretch in lignin [26], 1490 cm^{-1} - C=C and C-C stretching in bonds of lignin aromatic ring [27], 1740 cm^{-1} - C=O stretch of hemicellulose, 1745 cm^{-1} - C=O stretch in cellulose, lignin [28], 2266 cm^{-1} - O-H, C-O bonds in cellulose, 2332 cm^{-1} - C-H bond in cellulose [16,29] and 2923 cm^{-1} -C-H, C-H₂ stretch in cellulose [30]. Steam and alkali pretreatments brought about effective cellulosic delinearization as the obtained bands showed reduced intensities compared to the untreated samples. There were slight changes in band positions (from 1233 cm^{-1} to 1231 cm^{-1} for steam and 1230 cm^{-1} for sodium hydroxide. Untreated sweet lime fibers showed band peaks at 1205 cm^{-1} -O-H, C-O-C bond in cellulose [25], 1508 cm^{-1} -C=C stretch in lignin, 1740 cm^{-1} - C=O and C-H bond in hemicellulose [31], 1910 cm^{-1} - O-H stretch in cellulose, hemicellulose, and 2332 cm^{-1} - C-H bond in cellulose [29] and 2910 cm^{-1} -C-H bond stretch in cellulose, hemicellulose [32].

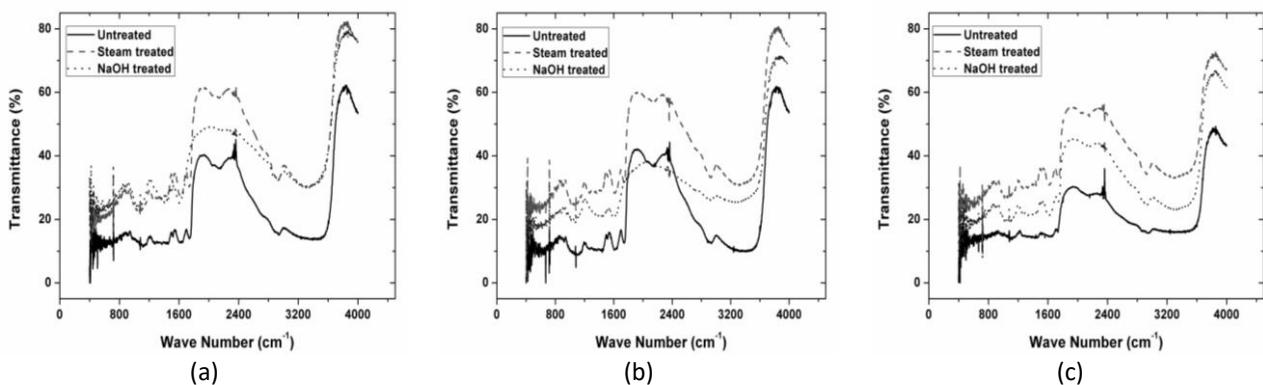


Fig. 2. FTIR Spectra of (a) orange peels (b) sweet lime peels (c) banana peels

The pretreated fibers resulted in the reduction of weaker bands and shift from their initial positions: (from 1205 cm^{-1} to 1209 cm^{-1} for steam and sodium hydroxide. The absence of a peak at 1740 cm^{-1} after alkali treatment could be due to irreversible structural modification to the existing C-O and C-H linking. The untreated orange fibers yielded the closest corresponding peaks: 1200 cm^{-1} -O-H, C-O-C bond stretch in cellulose [25], 1508 cm^{-1} -C=C vibrations in lignin [33], 1633 cm^{-1} - C=C bond in lignin [34] and 2970 cm^{-1} - C-H stretch in cellulose [25].

3.2 Characterization of Bioethanol

3.2.1 pH analysis

Saccharomyces boulardii used in this study successfully converted cellulosic content in fruit peels to ethanol. There was a steady increase in pH of biomass during fermentation involving both acidic and enzymatic hydrolysis of fruit peels as seen in (Table 2). The individual peels showed a periodic increase in pH of biomass (after acid hydrolysis): (3.11 to 5.12 for orange), (3.38 to 5.20 for sweet lime) and (3.25 to 5.06 for banana) after 120 h of fermentation. As a result of enzymatic hydrolysis, the pH changed from (4.51 to 5.58), (4.81 to 5.82) and (3.98 to 5.44) for orange, sweet lime, and banana respectively. It is well known that the decrease in pH is indicative of a gradual increase in bioethanol production. This is due to the production of organic acids during yeast-based anaerobic fermentation which reduces the overall pH of medium. Therefore, a lower pH during fermentation suggests ethanol as one of the accumulated final products [35]. This was seen also in the case of bioethanol produced from fruit (orange) and vegetable (potato, tomato, and cabbage) wastes. The tomato derived bioethanol pH decreased from 4.19 to 3.79, while, for potato, pH declined from 5.39 to 4.62 and for cabbage, it reduced from 5.35 to 4.19 [36]. Yeast isolate from sugarcane juice showed an increase in bioethanol yields from 22.4 g/L at pH 6 to 33.4 g/L at pH 5 when glucose was used as a defined sugar medium [37].

Table 2
pH during fermentation of bioethanol

Fruit	Time (h)	pH during ethanol fermentation	
		(acidic hydrolysis)	(enzymatic hydrolysis)
Orange	0	6.95±0.02	6.90±0.10
	24	3.11±0.16	4.51±0.05
	48	3.78±0.51	4.78±0.07
	72	4.13±0.20	4.94±0.34
	96	4.88±0.16	5.20±0.38
	120	5.12±0.13	5.58±0.43
Sweet Lime	0	6.87±0.05	6.92±0.14
	24	3.25±0.02	3.98±0.01
	48	3.82±0.05	4.29±0.08
	72	4.36±0.12	4.73±0.26
	96	4.81±0.09	5.10±0.16
	120	5.06±0.21	5.44±0.30
Banana	0	6.86±0.09	6.90±0.11
	24	3.38±0.36	4.81±0.18
	48	3.95±0.10	5.27±0.05
	72	4.21±0.14	5.45±0.19
	96	4.92±0.08	5.65±0.07
	120	5.20±0.11	5.82±0.27

3.2.2 Effect of hydrolysis on ethanol yield

Enzymatic hydrolysis of pre-treated fruit peels resulted in varying bioethanol yields for different fruit peels (Table 3). The individual peels resulted in a final yield of 3.9 mL, 4.2 mL, and 6.7 mL (for banana, orange, sweet lime) after sulfuric acid treatment as compared to 8.2 mL, 9.6 mL, and 12.1 mL for the same in the presence of α -amylase. After initial enzymatic hydrolysis, the partially delinearized fibers were converted to bioethanol with the help of microbial cellulases and hemicellulases produced by yeast *S. boulardii*. SHF also resulted in 228 mL of bioethanol at a larger

scale. Similar higher bioethanol yields were reported for sugarcane bagasse [1], where cellulase-hemicellulase combination resulted in effective biomass conversion as compared to dilute sulfuric acid. Several additional commercial enzymes such as β -glucosidases and endo-glucanases, exo-glucanases are also used for lignocellulosic conversion [38] as they are thermostable, highly specific and result in complete degradation of cellulose, hemicellulose into glucose and xylose, arabinose, mannose, galactose respectively without accumulation of byproducts [18]. These sugars are then converted to ethanol during anaerobic fermentation by microbial enzymes [39]. The increased surface area of the substrate due to enzymatic hydrolysis might be another reason behind higher ethanol production [40]. Acidic treatment resulted in a lower yield of ethanol which might be due to several factors. Acid hydrolysis is more suitable for substrates with high lignin content as it can break down its complex structure [41]. The treatment being non-specific to cellulose also break downs hemicellulose which blocks the accessibility of cellulose for further enzymatic degradation [42,43]. The acidic reaction in addition to being expensive also results in the incomplete breakdown of complex sugars into oligomers. Therefore, it forms inhibitory intermediates (such as furfurals, acetate, benzaldehydes) which interfere with fermentation and slows down the hydrolysis. This process necessitates neutralization which may lead to corrosive reactions [44].

3.2.3 Effect of fermentation time on bioethanol yield

An increase in fermentation time after 24 h resulted in lesser bioethanol yield as seen in Table 2. For orange peels subjected to acid and enzymatic hydrolysis, the bioethanol yields decreased from 4.2 mL and 9.6 mL after 24 h to 1.7 mL and 4.2 mL after 120 h. The sweet lime peels derived bioethanol yields declined from 6.7 mL and 12.1 mL to 2.6 mL and 6.8 mL. In the case of banana peels, the bioethanol yields reduced from 3.9 mL and 8.2 mL to 0.8 mL and 3 mL. The bioethanol yields increase with fermentation time up to a saturation point, after which it declines. This is the time when complex sugars in substrates (fruit peels) are completely converted to bioethanol by microbial enzymes. In the present study, 24 h was the point where bioethanol yield was the highest. In similar cases, bioethanol yield from citrus waste and banana peels declined from 26.84 g/L after 48 h to 25.22 g/L after 72 h [45]. Also, bioethanol from pineapple peels reduced from 5.98 g/L after to 4.5 g/L after 72 h [46].

Table 3
 Effect of hydrolysis on bioethanol production

Fruit peel type	Time (h)	Volume of produced bioethanol (mL)	
		Acidic hydrolysis	Enzymatic hydrolysis
Orange	24	4.2	9.6
	48	3.8	8.5
	72	3.3	6.4
	96	2.8	5.6
	120	1.7	4.2
Sweet Lime	24	6.7	12.1
	48	5.4	11.2
	72	4.8	10.5
	96	3.9	8.9
	120	2.6	6.8
Banana	24	3.9	8.2
	48	3.2	7.1
	72	2.5	6.3
	96	1.6	4.8
	120	0.8	3.0

3.2.4 Physiochemical properties of bioethanol

The bioethanol obtained from fruit peels showed optimum characteristics for use as automobile fuel. The physical properties of bioethanol were compared with that of absolute alcohol (Commercial ethanol) and gasoline in Table 4. The variation could be attributed to moisture content retention in the produced bioethanol after dehydration [47,48]. The produced bio-ethanol has been shown in Figure 3 for different sources of the fruit peels.



Fig. 3. Synthesized Bio-ethanol from different fruit peels (a) Banana (b) Orange (c) Lime

Table 4

Some Physiochemical properties of ethanol and gasoline [6,49]

Properties	Absolute Alcohol	Produced bioethanol	Gasoline
Chemical Formula	C ₂ H ₅ OH	C ₂ H ₅ OH	C ₈ H ₁₇
Molecular Weight	46	46	113.2
Specific gravity	0.79	0.83	0.74
Absolute Viscosity (cp)	1.2	1.31	0.56
Flash point (K)	290.15	302.15	228
Fire point (K)	299.15	307.15	234
Lower Heating Value (MJ/kg)	27	23.4	43.5

3.2.5 Gas Chromatographical (GC) analysis

Commercial ethanol was used as a reference for GC analysis as shown in Figure 4. The compound eluted from the column at a retention time of 4.26 min. The bioethanol obtained from fruit peels showed a retention time of 4.63 min with a deviation in retention time of 8.7 %, similar to results obtained in [50].

3.3 Performance Testing

The variation in TFC with BMEP at an engine speed of 2000 rpm has been displayed in Figure 5(a). With an increase in BMEP, the fuel consumption was found to increase for each blend. At any given BMEP, E0 showed the maximum fuel consumption and as the bioethanol content increased in the blend, the total fuel consumption was found to drop. BSFC with BMEP at an engine speed of 2000 rpm has been shown in Figure 5(b). With the increase in BMEP, the BSFC was found to decrease, in all the four blends. At a given BMEP, the BSFC was found to decrease as ethanol content was increased. Thus, bioethanol showed a reducing effect on the BSFC. The brake thermal efficiency was found to an increase with an increase in BMEP as well as bioethanol content (Figure 6(a)). The peak efficiency of 16.8% was noticed for the E12 bioethanol blend. The thermal efficiency could be improved by increasing the compression ratio which could be accommodated for bioethanol blends since ethanol has a higher octane number than gasoline [47]. The volumetric efficiency increased on increasing the BMEP for all the blends (Figure 6(b)). The increase in bioethanol content enhanced the volumetric efficiency. At the peak BMEP, the effect of bioethanol content on the volumetric efficiency was found to be more pronounced for the E12 blend.

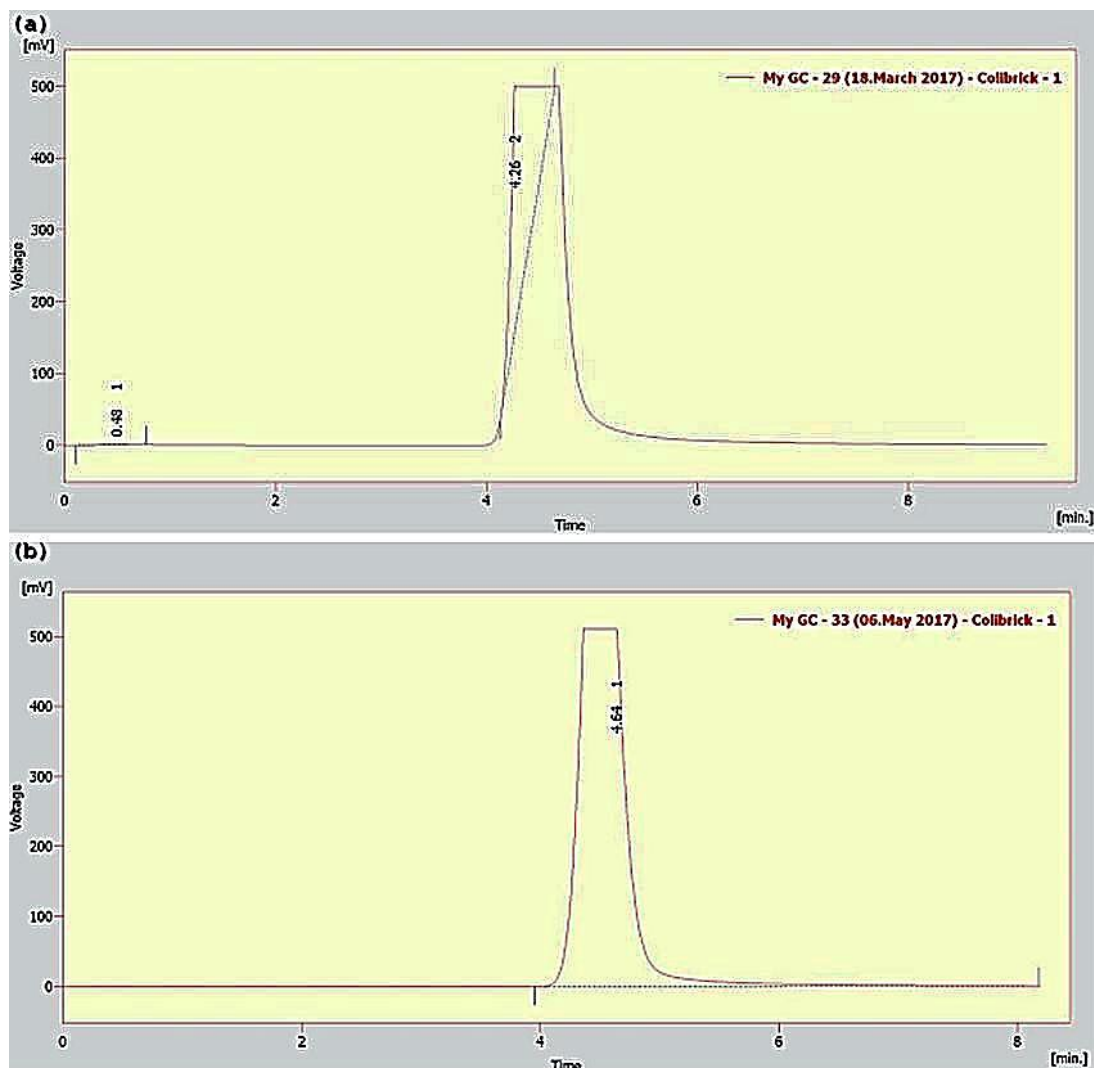


Fig. 4. GC Analysis (a) Absolute alcohol (b) Bioethanol

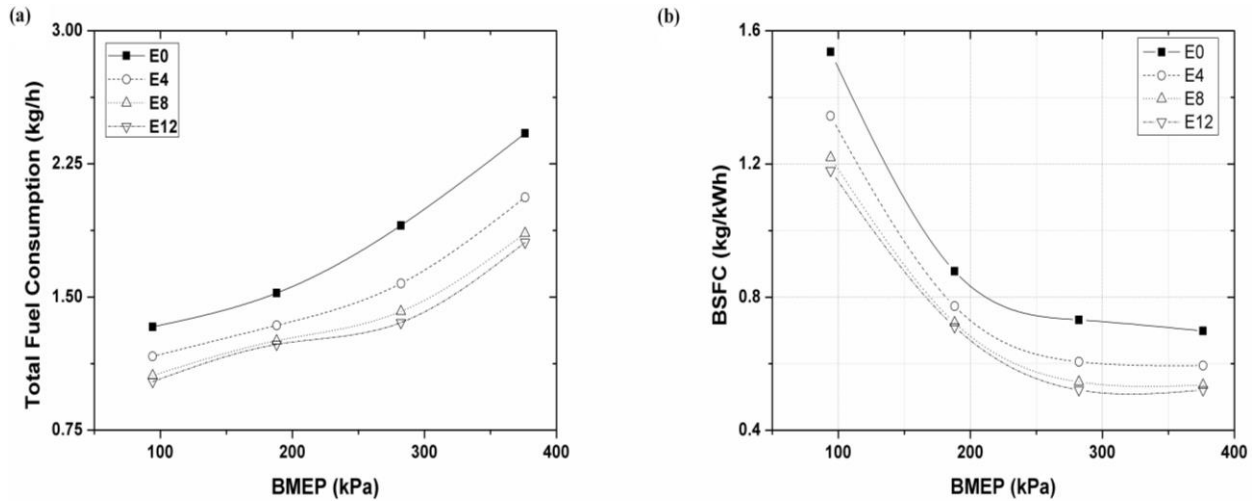


Fig. 5. (a) TFC Vs BMEP @ 2000 RPM (b) BSFC Vs BMEP @ 2000 RPM

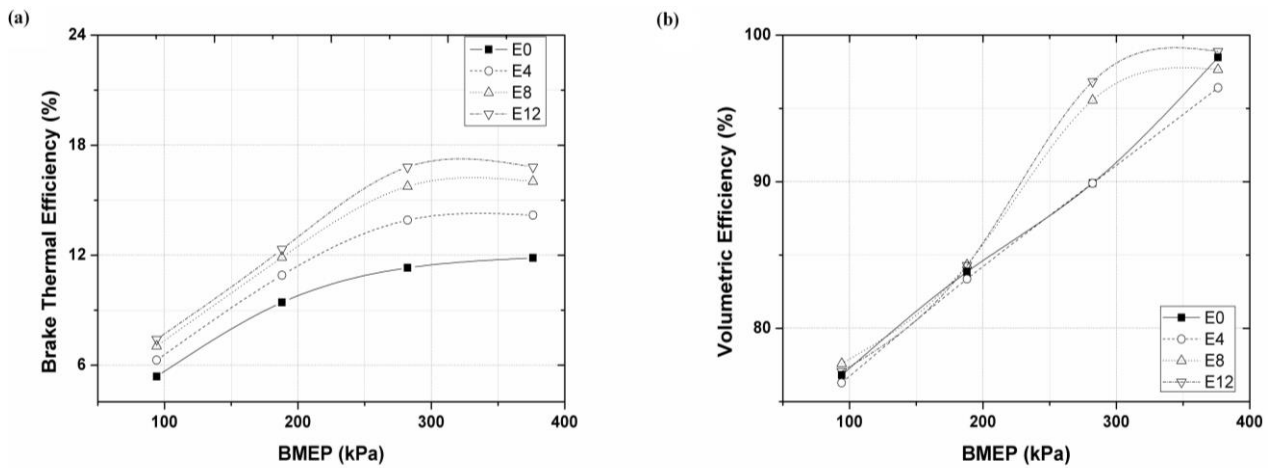


Fig. 6. (a) BTE Vs BMEP @ 2000 RPM (b) Volumetric Efficiency Vs BMEP @ 2000 RPM

Figure 7 shows the variation of the equivalence ratio with the BMEP at an engine speed of 2000 rpm. The equivalence ratio of E0 i.e. 100% gasoline which lay within lean limits at the lowest BMEP increased with BMEP and the mixture attained At-Stoichiometric strength at the peak BMEP. The bioethanol blends E4, E8 and E12 showed an equivalence ratio less than unity i.e. the mixtures remained lean at all values of the BMEP. E8 and E12 showed very lean mixtures at the lowest BMEP, hence posing a risk of premature flame quenching at cold start conditions [51,52].

Hence, for increasing the content of bioethanol beyond 12%, 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 shown in Table 5 along with the density and lower heating values of different blends.

Table 5
 Comparison of operational parameters of different blends

Blend type	$(A/F)_{Ac}$ Engine load 25%	Engine load 50%	Engine load 75%	Engine load 100%	$(A/F)_s$	ρ_f (kg/m ³)	Q_{cv} (MJ/kg)
E0	21.6	20.6	17.7	15.2	14.94	740	43.5
E4	24.5	23.3	21.4	17.5	14.76	745	42.7
E8	27.5	25.2	25.2	19.7	14.58	749	41.9
E12	28.2	25.6	26.8	20.5	14.39	753	41.1

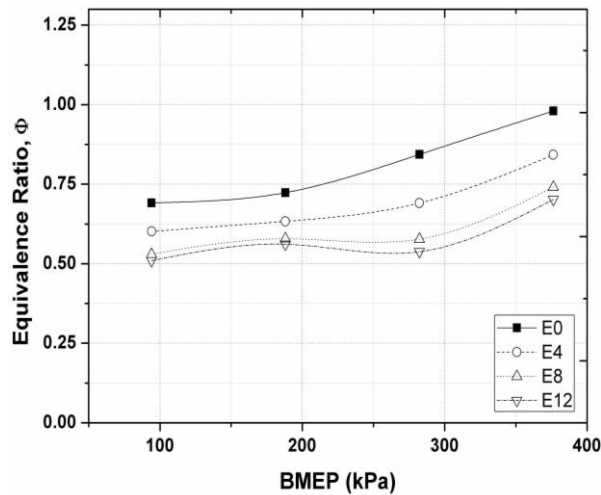


Fig. 7. Equivalence Ratio Vs BMEP

4. Conclusions

The cellulosic content in banana, sweet lime and orange were successfully converted into bioethanol by the action of α -amylase treatment and cellulases produced by *Saccharomyces boulardii*. Both steam and alkali pretreatments with sodium hydroxide brought about significant changes in the lignocellulosic structure which were analyzed using FTIR spectra analysis. Sweet lime peel resulted in a maximum yield of ethanol after enzymatic hydrolysis as compared to dilute sulfuric acid. α -amylase proved to be a potential replacement for the commercially expensive cellulases in bio-based fuel production. GC analysis confirmed the authenticity of the produced bioethanol. The obtained bioethanol showed effective physiochemical properties requisite for application in automobile fuels.

The following conclusions were drawn from the performance testing of the bioethanol blends with gasoline on the four-stroke, spark-ignition engine:

- i. The brake thermal efficiency increased with an increase in ethanol content as well as an increase in BMEP's.
- ii. TFC increased while BSFC reduced with the increasing ethanol content at different BMEP's.
- iii. The volumetric efficiency was found to rise with the ethanol content at different BMEP's.
- iv. 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.

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